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(54) PROCEDE POUR AUGMENTER LA TENEUR EN HUILE DANS DES VEGETAUX
(54) METHODS FOR INCREASING OIL CONTENT IN PLANTS

(57)

The invention relates to methods for increasing the oil content in plants, preferably in the seeds of plants, by expression of glycerol-3-phosphatdehydrogenases (G3PDH) from yeast, preferably from Saccharomyces cerevisiae. The invention also relates to expression constructs for the expression of G3PDH yeast in plants, preferably in the seeds of plants, transgenic plants expressing G3PDH, and to the use of said transgenic plants in the production of foodstuffs, feed, seeds, pharmaceuticals or fine chemicals, especially in the production of oils.

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The invention relates to methods for increasing the oil content in plants, preferably in the seeds of plants, by expression of glycerol-3-phosphatdehydrogenases (G3PDH) from yeast, preferably from Saccharomyces cerevisiae. The invention also relates to expression constructs for the expression of G3PDH yeast in plants, preferably in the seeds of plants, transgenic plants expressing G3PDH, and to the use of said transgenic plants in the production of foodstuffs, feed, seeds, pharmaceuticals or fine chemicals, especially in the production of oils.

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(54) Title: METHODS FOR INCREASING OIL CONTENT IN PLANTS

(54) Bezeichnung: VERFAHREN ZUM ERHÖHEN DES ÖLGEHALTES IN PFLANZEN

(57) Abstract: The invention relates to methods for increasing the oil content in plants, preferably in the seeds of plants, by expression of glycerol-3-phosphatdehydrogenases (G3PDH) from yeast, preferably from Saccharomyces cerevisiae. The invention also relates to expression constructs for the expression of G3PDH yeast in plants, preferably in the seeds of plants, transgenic plants expressing G3PDH, and to the use of said transgenic plants in the production of foodstuffs, feed, seeds, pharmaceuticals or fine chemicals, especially in the production of oils.

(57) Zusammenfassung: Die Erfindung betrifft Verfahren zum Erhöhen des Ölgehaltes in Pflanzen, bevorzugt in pflanzlichen Samen, durch Expression von Glycerol-3-phosphatdehydrogenasen (G3PDH) aus Hefen, bevorzugt aus Saccharomyces cerevisiae. Die Erfindung betrifft ferner Expressionskonstrukte zur Expression von Hefe G3PDH in Pflanzen, bevorzugt in pflanzlichen Samen, transgene Pflanzen exprimierend Hefe G3PDH, sowie die Verwendung von besagter transgener Pflanzen zur Herstellung von Nahrungs-, Futtermitteln, Saatgut, Pharmazeutika oder Feinchemikalien, insbesondere zur Herstellung von Ölen.

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METHODS FOR INCREASING OIL CONTENT IN PLANTS

The invention relates to methods for increasing the oil content in plants, preferably in plant seeds, by expressing yeast glycerol-3-phosphate dehydrogenases (G3PDH), preferably from Saccharomyces cerevisiae. The invention furthermore relates to expression constructs for expressing yeast G3PDH in plants, preferably in plant seeds, transgenic plants expressing yeast G3PDH, and to the use of said transgenic plants for the production of food, feeds, seed, pharmaceuticals or fine chemicals, in particular for the production of oils.

Increasing the oil content in plants and, in particular, in plant seeds is of great interest for traditional and modern plant breeding and in particular for plant biotechnology. Owing to the increasing consumption of vegetable oils for nutrition or industrial applications, possibilities of increasing or modifying vegetable oils are increasingly the subject of current research (for example Töpfer et al. (1995) Science 268:681-686). Its aim is in particular increasing the fatty acid content in seed oils.

The fatty acids which can be obtained from the vegetable oils are also of particular interest. They are employed, for example, as bases for plasticizers, lubricants, surfactants, cosmetics and the like and are employed as valuable bases in the food and feed industries. Thus, for example, it is of particular interest to provide rapeseed oils with fatty acids with medium chain length since these are in demand in particular in the production of surfactants.

The targeted modulation of plant metabolic pathways by recombinant methods allows the modification of the plant metabolism in an advantageous manner which, when using traditional breeding methods, could only be achieved after a complicated procedure or not at all. Thus, unusual fatty acids, for example specific polyunsaturated fatty acids, are only synthesized in certain plants or not at all in plants and can therefore only be produced by expressing the relevant enzyme in transgenic plants (for example Millar et al. (2000) Trends Plant Sci 5:95-101).

Triacylgylcerides and other lipids are synthesized from fatty acids. Fatty acid biosynthesis and triacylglyceride biosynthesis can be considered as separate biosynthetic pathways owing to the compartmentalization, but as a single biosynthetic pathway in view of the end product. Lipid synthesis can be divided into two

part-mechanisms, one which might be termed "prokaryotic" and another which may be termed "eukaryotic" (Browse et al. (1986) Biochemical J 235:25-31; Ohlrogge & Browse (1995) Plant Cell 7:957-970). The prokaryotic mechanism is localized in the plastids and encompasses the biosynthesis of the free fatty acids which are exported into the cytosol, where they enter the eukaryotic mechanism in the form of fatty acid acyl-CoA esters and are esterified with glycerol-3-phosphate (G3P) to give phosphatidic acid (PA). PA is the starting point for the synthesis of neutral and polar lipids. The neutral lipids are synthesized on the endoplasmic reticulum via the Kennedy pathway (Voelker (1996) Genetic Engineering, Setlow (ed.) 18:111-113; Shankline & Cahoon (1998) Annu Rev Plant Physiol Plant Mol Biol 49:611-649; Frentzen (1998) Lipids 100:161-166). Besides the biosynthesis of triacylglycerides, G3P also plays a role in glycerol synthesis (for example for the purposes of osmoregulation and against low-temperature stress for example).

GP3, which is essential for the synthesis, is synthesized here by the reduction of dihydroxyacetone phosphate (DHAP) by means of glycerol-3-phosphate dehydrogenase (G3PDH), also termed dihydroxyacetone phosphate reductase. As a rule, NADH acts as reducing cosubstrate (EC 1.1.1.8). A further class of glycerol-3-phosphate dehydrogenases (EC 1.1.99.5) utilizes FAD as cosubstrate. The enzymes of this class catalyze the reaction of DHAP to G3P. In eukaryotic cells, the two classes of enzymes are distributed in different compartments, those which are NAD-dependent being localized in the cytosol and those which are FAD-dependent being localized in the mitochondria (for Saccharomyces cerevisiae, see, for example, Larsson et al., 1998, Yeast 14:347-357). EP-A 0 353 049 describes an NAD-independent G3PDH from Bacillus sp. In Saccharomyces cerevisiae too, an NAD-independent G3PDH is identified (Miyata K, Nagahisa M (1969) Plant Cell Physiol 10(3):635-643).

G3PDH is an essential enzyme in prokaryotes and eukaryotes which, besides having a function in lipid biosynthesis, is one of the enzymes responsible for maintaining the cellular redox status by acting on the NAD+/NADH ratio. Deletion of the GPD2 gene in Saccharomyces cerevisiae (one of two G3PDH isoforms in this yeast) results in reduced growth under anaerobic conditions. In addition, G3PDH appears to play a role in the stress response of yeast mainly to osmotic stress. Deletion of the GPD1 gene in Saccharomyces cerevisiae causes hypersensitivity to sodium chloride.

Sequences for G3PDHs have been described for insects (Drosophila melanogaster, Drosophila virilis), plants (Arabidopsis thaliana, Cuphea lanceolata), mammals (Homo sapiens, Mus musculus, Sus scrofa, Rattus norvegicus), fish (Salmo salar, Osmerus mordax), birds (Ovis aries), amphibians (Xenopus laevis), nematodes (Caenorhabditis elegans), algae and bacteria.

Plant cells have at least two G3PDH isoforms, a cytoplasmic isoform and a plastid isoform (Gee RW et al. (1988) Plant Physiol 86:98-103; Gee RW et al. (1988) Plant Physiol 87:379-383). In plants, the enxymatic activity of glycerol-3-phosphate dehydrogenase was first found in potato tubors (Santora GT et al. (1979) Arch Biochem Biophys 196:403-411). Further G3PDH activities which were localized in the cytosol and the plastids were detected in other plants such as peas, maize or soya (Gee RW et al. (1988) PLANT PHYSIOL 86(1): 98-103). G3PDHs from algae such as, for example, two plastid G3PDH isoforms and one cytosolic G3PDH isoform from Dunaliella tertiolecta have furthermore been described (Gee R et al.(1993) Plant Physiol 103(1):243-249; Gee R et al. (1989) PLANT PHYSIOL 91(1):345-351). As regards the plant G3PDH from Cuphea lanceolata, it has been proposed to obtain an increased oil content or a shift in the fatty acid pattern by overexpression in plants (WO 95/06733). However, such effects have not been proven.

Bacterial G3PDHs and their function have been described (Hsu and Fox (1970) J *Bacteriol* 103:410-416; Bell (1974) J *Bacterial* 117:1065-1076).

WO 01/21820 describes the heterologous expression of a mutated E. coli G3PDH for increased stress tolerance and modification of the fatty acid composition in storage oils. The muttated E.coli G3PDH (gpsA2FR) exhibits a single amino acid substitution which brings about reduced inhibition via G3P. The heterologous expression of the gpsA2FR mutant leads to glycerolipids with an increased C16 fatty acid content and, accordingly, a reduced C18 fatty acid content. The modifications in the fatty acid pattern are relatively minor: an increase of 2 to 5% in the 16:0 fatty acids and of 1.5 to 3.5% in the 16:3 fatty acids, and a reduction in 18:2 and 18:3 fatty acids by 2 to 5% were observed. The total glycerolipid content remained unaffected.

G3PDHs from yeasts (Ascomycetes) such as

a) Schizosaccharomyces pombe (Pidoux AL et al. (1990) Nucleic Acids Res 18 (23): 7145; GenBank Acc.-No.: X56162; Ohmiya R et al. (1995) Mol Microbiol 18(5):963-73; GenBank Acc.-No.: D50796, D50797),

b) Yarrowia lipolytica (GenBank Acc.-No.: AJ250328)

c) Zygosaccharomyces rouxii (Iwaki T et al. Yeast (2001) 18(8):737-44; GenBank Acc.-No: AB047394, AB047395, AB047397) or

d) Saccharomyces cerevisiae (Albertyn J et al. (1994) Mol Cell Biol 14(6):4135-44; Albertyn J et al. (1992) FEBS LETT 308(2):130-132; Merkel JR et al. (1982) Anal Biochem 122 (1):180-185; Wang HT et al. (1994) J Bacteriol. 176(22):7091-5; Eriksson P et al. (1995) Mol Microbiol. 17(1):95-107; GenBank Acc.-No.: U04621, X76859, Z35169).

e) Emericella nidulans (GenBank Acc.-No.: AF228340)

f) Debaryomyces hansenii (GenBank Acc.-No.: AF210060)

are furthermore described.

It is an object of the present invention to provide alternative methods for increasing the oil content in plants. We have found that this object is achieved by the present invention.

A first subject matter of the invention comprises a method of increasing the total oil content in a plant organism or a tissue, organ, part, cell or propagation material thereof, comprising

a) the transgenic expression of yeast glycerol-3-phosphate dehydrogenase in said plant organism or in a tissue, organ, part, cell or propagation material thereof, and

b) the selection of plant organisms in which - in contrast to or comparison with the starting organism - the total oil content in said plant organism or in a tissue, organ, part, cell or propagation material thereof is increased.

Surprisingly, it has been found that the seed-specific heterologous expression of the yeast protein Gpd1p (G3PDH from Saccharomyces cerevisiae; SEQ ID NO: 2) in Arabidopsis seeds leads to a significantly increased triacylglyceride (storage

oils) content. The oil content was increased by approximately 22%, in a transgenic line even by 41%, compared with wild-type control plants (see Fig. 1). The transgenic expression of the yeast glycerol 3-phosphate dehydrogenase had no adverse effects on the growth or other properties of the transformed plants. Since G3PDH is a biosynthetic key enzyme in all plant organisms, the method according to the invention can be applied in principle to all plant species, in addition to the species *Arabidopsis thaliana*, which is employed as model plant. The method according to the invention is preferably applied to oil crops whose oil content is already naturally high and/or for the industrial production of oils.

"Plant" organism or tissue, organ, part, cell or propagation material thereof" is generally understood as meaning any single- or multi-celled organism or a cell, tissue, part or propagation material (such as seeds or fruit) of same which is capable of photosynthesis. Included for the purpose of the invention are all genera and species of higher and lower plants of the Plant Kingdom. Annual, perennial, monocotyledonous and dicotyledonous plants are preferred. Also included are mature plants, seeds, shoots and seedlings, and parts, propagation material (for example tubors, seeds or fruits) and cultures derived from them, for example cell cultures or callus cultures.

For the purposes of the invention, "plant" refers to all genera and species of higher and lower plants of the Plant Kingdom. The term includes the mature plants, seeds, shoots and seedlings, and parts, propagation material, plant organ tissue, protoplasts, callus and other cultures, for example cell cultures, derived from them, and all other species of groups of plant cells giving functional or structural units. Mature plants refers to plants at any developmental stage beyond the seedling. Seedling refers to a young, immature plant at an early developmental stage.

"Plant" encompasses all annual and perennial monocotyldedonous or dicotyledonous plants and includes by way of example, but not by limitation, those of the genera Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solarium, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis,

Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Picea and Populus.

Preferred plants are those from the following plant families: Amaranthaceae, Asteraceae, Brassicaceae, Carophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Labiatae, Leguminosae, Papilionoideae, Liliaceae, Linaceae, Malvaceae, Rosaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Solanaceae, Sterculiaceae, Tetragoniaceae, Theaceae, Umbelliferae.

Preferred monocotyledonous plants are selected in particular from the monocotyledonous crop plants such as, for example, the Gramineae family, such as rice, maize, wheat or other cereal species such as barley, millet and sorghum, rye, triticale or oats, and sugar cane, and all grass species.

The invention is applied very particularly preferably from dicotyledonous plant organisms. Preferred dicotyledonous plants are selected in particular from the dicotyledonous crop plants such as, for example,

- Asteraceae such as sunflower, tagetes or calendula and others,
- Compositae, especially the genus Lactuca, very particularly the species sativa (lettuce) and others,
- Cruciferae, particularly the genus Brassica, very particularly the specis napus (oilseed rape), campestris (beet), oleracea cv Tastie (cabbage), oleracea cv Snowball Y (cauliflower) and oleracea cv Emperor (broccoli) and other cabbages; and the genus Arabidopsis, very particularly the species thaliana, and cress or canola and others,
- Cucurbitaceae such as melon, pumpkin/squash or zucchini and others,
- Leguminosae, particularly the genus Glycine, very particularly the species max (soybean), soya, and alfalfa, pea, beans or peanut and others,
- Rubiaceae, preferably the subclass Lamiidae such as, for example Coffea arabica or Coffea liberica (coffee bush) and others,
- Solanaceae, particularly the genus Lycopersicon, very particularly the species esculentum (tomato), the genus Solanum, very particularly the species tuberosum (potato) and

melongena (aubergine) and the genus Capsicum, very particularly the genus annuum (pepper) and tobacco or paprika and others,

- Sterculiaceae, preferably the subclass Dilleniidae such as, for example, Theobroma cacao (cacao bush) and others,

- Theaceae, preferably the subclass Dilleniidae such as, for example, Camellia sinensis or Thea sinensis (tea shrub) and others,

- Umbelliferae, particularly the genus Daucus (very particularly the species carota (carrot)) and Apium (very particularly the species graveolens dulce (celery)) and others;

and linseed, cotton, hemp, flax, cucumber, spinach, carrot, sugar beet and the various tree, nut and grapevine species, in particular banana and kiwi fruit.

Also encompassed are ornamental plants, useful or ornamental trees, flowers, cut flowers, shrubs or turf. Plants which may be mentioned by way of example but not by limitation are angiosperms, bryophytes such as, for example, Hepaticae (liver flowers) and Musci (mosses); pteridophytes such as ferns, horsetail and clubmosses; gymnosperms such as conifers, cycads, ginkgo and Gnetatae, the families of the Rosaceae such as rose, Ericaceae such as rhododendron and azalea, Euphorbiaceae such as poinsettias and croton, Caryophyllaceae such as pinks, Solanaceae such as petunias, Gesneriaceae such as African violet, Balsaminaceae such as touch-me-not, Orchidaceae such as orchids, Iridaceae such as gladioli, iris, freesia and crocus, Compositae such as marigold, Geraniaceae such as geranium, Liliaceae such as dracena, Moraceae such as ficus, Araceae such as cheeseplant and many others.

Furthermore, plant organisms for the purposes of the invention are further organisms capable of being photosynthetically active such as, for example, algae, cyanobacteria and mosses. Preferred algae are green algae such as, for example, algae from the genus Haematococcus, Phaedactylum tricornatum, Volvox or Dunaliella. Synechocystis is particularly preferred.

Most preferred are oil crops. Oil crops are understood as being plants whose oil content is already naturally high and/or which can be used for the industrial production of oils. These plants can have a high oil content and/or else a particular fatty acid composition which is of interest industrially. Preferred plants

are those with a lipid content of at least 1% by weight. Oil crops encompass by way of example: Borago officinalis (borage); Brassica species such as *B. campestris, B. napus, B. rapa* (mustard, oilseed rape or turnip rape); *Cannabis sativa* (hemp); Carthamus *tinctorius* (safflower); Cocos *nucifera* (coconut); Crambe abyssinica (crambe); Cuphea species (Cuphea species yield fatty acids of medium chain length, in particular for industrial applications); *Elaeis guinensis* (African oil palm); *Elaeis oleifera* (American oil palm); *Glycine max* (soybean); *Gossypium hirsutum* (American cotton); *Gossypium barbadense* (Egyptian cotton); *Gossypium herbaceum* (Asian cotton); *Helianthus annuus* (sunflower); *Linum usitatissimum* (linseed or flax); *Oenothera biennis* (evening primrose); Olea *europaea* (olive); Oryza *sativa* (rice); *Ricinus communis* (castor); Sesamum *indicum* (sesame); *Triticum* species (wheat); Zea *mays* (maize), and various nut species such as, for example, walnut or almond.

"Total oil content" refers to the sum of all oils, preferably to the sum of the triacylglycerides.

"Oils" encompasses neutral and/or polar lipids and mixtures of these. Those mentioned in Table 1 may be mentioned by way of example, but not by limitation.

Table 1: Classes of plant lipids

Neutral lipids	Triacylglycerol (TAG)
	Diacylglycerol (DAG)
	Monoacylglycerol (MAG)
Polar lipids	Monogalactosyldiacylglycerol (MGDG)
	Digalactosyldiacylglycerol (DGDG)
	Phosphatidylglycerol (PG)
	Phosphatidylcholine (PC)
	Phosphatidylethanolamine (PE)
	Phosphatidylinositol (PI)
	Phosphatidylserine (PS)
	Sulfoquinovosyldiacylglycerol

Neutral lipids preferably refers to triacylglycerides. Both neutral and polar lipids may comprise a wide range of various fatty acids. The fatty acids mentioned in Table 2 may be mentioned by way of example, but not by limitation.

Table 2: Overview over various fatty acids (selection)
[1] Chain length: number of double bonds
* not naturally occurring in plants

Nomenclature[1]	Name
16:0	Palmitic acid
16:1	Palmitoleic acid
16:3	Roughanic acid
18:0	Stearic acid
18:1	Oleic acid
18:2	Linoleic acid
18:3	Linolenic acid
γ-18:3	Gamma-linolenic acid*
20:0	Arachidic acid
22:6	Docosahexanoic acid (DHA) *
20:2	Eicosadienoic acid
20:4	Arachidonic acid (AA) *
20:5	Eicosapentaenoic acid (EPA) *
22:1	Erucic acid

Oils preferably relates to seed oils.

"Increase in" the total oil content refers to the increased oil content in a plant or a part, tissue or organ thereof, preferably in the seed organs of the plants. In this context, the oil content is at least 5%, preferably at least 10%, particularly preferably at least 15%, very particularly preferably at least 20%, most preferably at least 25% increased under otherwise identical conditions in comparison with a starting plant which has not been subjected to the method according to the invention, but is otherwise unmodified. Conditions in this context means all of the conditions which are relevant for germination, culture or growth of the plant, such as soil conditions, climatic conditions, light conditions, fertilization, irrigation, plant protection treatment and the like.

"Yeast glycerol 3-phosphate dehydrogenase" (termed "yeast G3PDH" hereinbelow) generally refers to all those enzymes which are capable of converting dihydroxyacetone phosphate (DHAP) into glycerol-3-phosphate (G3P) - preferably using a cosubstrate such as NADH - and which are naturally expressed in a yeast.

Yeast refers to the group of unicellular fungi with a pronounced cell wall and formation of pseudomycelium (in contrast to molds). They reproduce vegetatively by budding and/or fission (Schizosaccharomyces and Saccharomycodes, respectively).

Encompassed are what are known as false yeasts, preferably the families Cryptococcaceae, Sporobolomycetaceae with the genera Cryptococcus, Torulopsis, Pityrosporum, Brettanomyces, Candida, Kloeckera, Trigonopsis, Trichosporon, Rhodotorula and Sporobolomyces and Bullera, and true yeasts (yeasts which also reproduce sexually; ascus), preferably the families endo- and saccharomycetaceae, with the genera Saccharomyces, Debaromyces, Lipomyces, Hansenula, Endomycopsis, Pichia, Hanseniaspora. Most preferred are the genera Saccharomyces cerevisiae, Pichia pastoris, Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Zygosaccharomyces rouxii, Yarrowia lipolitica, Emericella nidulans, Aspergillus nidulans, Debaryomyces hansenii and Torulaspora hansenii.

Yeast G3PDH refers in particular to polypeptides which have the following characteristics as "essential characteristics":

a) the conversion of dihydroxyacetone phosphate into glycerol-3-phosphate using NADH as cosubstrate (EC 1.1.1.8), and

b) a peptide sequence encompassing at least one sequence motif selected from the group of sequence motifs consisting of

i)	GSGNWGT(A/T)IAK	(SEQ ID NO: 22)
ii)	CG(V/A)LSGAN(L/I/V)AXE(V/I)A	(SEQ ID NO: 26)
iii)	(L/V)FXRPYFXV	(SEQ ID NO: 27)

preferred is the sequence motif selected from the group consisting of

iv)	GSGNWGTTIAKV(V/I)AEN	(SEQ ID NO: 29)
v)	NT(K/R)HQNVKYLP	(SEQ ID NO: 30)
vi)	D(I/V)LVFN(I/V)PHQFL	(SEQ ID NO: 31)
vii)	RA(I/V)SCLKGFE	(SEQ ID NO: 32)
viii)	CGALSGANLA(P/T)EVA	(SEQ ID NO: 33)
ix)	LFHRPYFHV	(SEQ ID NO: 34)
x)	GLGEII(K/R)FG	(SEQ ID NO: 35)

the peptide sequence particularly preferably comprises at least 2 or 3, very particularly preferably at least 4 or 5, most preferably all of the sequence motifs selected from the group of the sequence motifs i), ii) and iii) or selected from the group of the sequence motifs iv), v), vi), vii), viii), ix) and xiv). (Terms in brackets refer to amino acids which are possible at this position as alternatives; for

example (V/I) means that valin or isoleucin are possible at this position).

Moreover, a yeast G3PDH may optionally comprise - in addition to at least one of the abovementioned sequence motifs i) to x) - further sequence motifs selected from the group consisting of

xi) H(E/Q)NVKYL (SEQ ID NO: 23)
xii) (D/N)(I/V)(L/I)V(F/W)(V/N)(L/I/V)PHQF(V/L/I) (SEQ ID NO: 24)
xiii) (A/G)(I/V)SC(L/I)KG (SEQ ID NO: 25)
xiv) G(L/M)(L/G)E(M/I)(I/Q)(R/K/N)F(G/S/A) (SEQ ID NO: 28)

Most preferably, yeast G3PDH refers to the yeast protein Gpd1p as shown in SEQ ID NO: 2 and functional equivalents or else functionally equivalent portions of the above.

Functional equivalents refers in particular to natural or artificial mutations of the yeast protein Gpd1p as shown in SEQ ID NO: 2 and homologous polypeptides from other yeasts which have the same essential characteristics of a yeast G3PDH as defined above. Mutations encompass substitutions, additions, deletions, inversions or insertions of one or more amino acid residues. Especially preferred are the polypeptides described by SEQ ID NO: 4, 5, 7, 9, 11, 12, 14, 16, 38 or 40.

The yeast G3PDH to be employed advantageously within the scope of the present invention can be found readily by database searches or by screening gene or cDNA libraries using the yeast G3PDH sequence shown in SEQ ID NO: 2, which is given by way of example, or the nucleic acid sequence as shown in SEQ ID NO: 1, which encodes the latter, as search sequence or probe.

Said functional equivalents preferably have at least 60%, particularly preferably at least 70%, particularly preferably at least 80%, most preferably at least 90% homology with the protein with the SEQ ID NO: 2.

Homology between two polypeptides is understood as meaning the identity of the amino acid sequence over the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 8 Length Weight: 2

Average Match: 2,912 Average Mismatch: -2,003

For example, a sequence with at least 80% homology with the sequence SEQ ID NO: 2 at the protein level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 2 with the above program algorithm and the above parameter set has at least 80% homology.

Functional equivalents also encompasses those proteins which are encoded by nucleic acid sequences which have at least 60%, particularly preferably at least 70%, particularly preferably at least 80%, most preferably at least 90% homology with the nucleic acid sequence with the SEQ ID NO: 1.

Homology between two nucleic acid sequences is understood as meaning the identity of the two nucleic acid sequences over the entire sequence length which is calculated by comparison with the aid of the program algorithm GAP (Wisconsin Package Version 10.0, University of Wisconsin, Genetics Computer Group (GCG), Madison, USA), setting the following parameters:

Gap Weight: 50 Length Weight: 3

Average Match: 10 Average Mismatch:0

For example, a sequence which has at least 80% homology with the sequence SEQ ID NO: 1 at the nucleic acid level is understood as meaning a sequence which, upon comparison with the sequence SEQ ID NO: 1 with the above program algorithm with the above parameter set has a homology of at least 80%.

Functional equivalents also encompass those proteins which are encoded by nucleic acid sequences which hybridize under standard conditions with a nucleic acid sequence described by SEQ ID NO: 1, the nucleic acid sequence which is complementary thereto or parts of the above and which have the essential characteristics for a yeast G3PDH.

"Standard hybridization conditions" is to be understood in the broad sense, but preferably refers to stringent hybridization conditions. Such hybridization conditions are described, for example, by Sambrook J, Fritsch EF, Maniatis T et al., in Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory Press, 1989, pages 9.31-9.57) or in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the conditions during the wash step can

be selected from the range of high-stringency conditions (with approximately 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3 M sodium citrate, 3 M NaCl, pH 7.0). Denaturing agents such as, for example, formamide or SDS may also be employed during hybridization. In the presence of 50% formamide, hybridization is preferably carried out at 42°C.

The invention furthermore relates to transgenic expression constructs which can ensure a transgenic expression of a yeast G3PDH in a plant organism or a tissue, organ, part, cells or propagation material of said plant organism.

The definition given above applies to yeast G3PDH, with the transgenic expression of a yeast G3PDH described by the sequence with the SEQ ID NO: 2 being particularly preferred.

In said transgenic expression constructs, a nucleic acid molecule encoding a yeast G3PDH is preferably in operable linkage with at least one genetic control element (for example a promoter) which ensures expression in a plant organism or a tissue, organ, part, cell or propagation material of same.

Especially preferred are transgenic expression cassettes wherein the nucleic acid sequence encoding a glycerol-3-phosphate dehydrogenase is described by

a) a sequence with the SEQ ID NO: 1, 3, 6, 8, 10, 13, 15, 37 or 39, or

b) a sequence derived from a sequence with the SEQ ID NO: 1, 3, 6, 8, 10, 13, 15, 37 or 39 in accordance with the degeneracy of the genetic code

c) a sequence which has at least 60% identity with the sequence with the SEQ ID NO: 1.

Operable linkage is understood as meaning, for example, the sequential arrangement of a promoter with the nucleic acid sequence encoding a yeast G3PDH which is to be expressed (for example the sequence as shown in SEQ ID NO: 1) and, if appropriate, further regulatory elements such as, for example, a terminator in such a way that each of the regulatory elements can fulfil its function when the nucleic acid sequence is expressed recombinantly. Direct linkage in the chemical sense is not necessarily required for this purpose. Genetic control sequences such as, for example, enhancer sequences can also exert their function on the target sequence from positions which are further

removed or indeed from other DNA molecules. Preferred arrangements are those in which the nucleic acid sequence to be expressed recombinantly is positioned behind the sequence acting as promoter so that the two sequences are linked covalently to each other. The distance between the promoter sequence and the nucleic acid sequence to be expressed recombinantly is preferably less than 200 base pairs, particularly preferably less than 100 base pairs, very particularly preferably less than 50 base pairs.

Operable linkage and a transgenic expression cassette can both be effected by means of conventional recombination and cloning techniques as they are described, for example, in Maniatis T, Fritsch EF and Sambrook J (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Silhavy TJ, Berman ML und Enquist LW (1984) Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor (NY), in Ausubel FM et al. (1987) Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience and in Gelvin et al. (1990) In: Plant Molecular Biology Manual. However, further sequences which, for example, act as a linker with specific cleavage sites for restriction enzymes, or of a signal peptide, may also be positioned between the two sequences. Also, the insertion of sequences may lead to the expression of fusion proteins. Preferably, the expression cassette composed of a promoter linked to a nucleic acid sequence to be expressed can be in a vector-integrated form and can be inserted into a plant genome, for example by transformation.

However, a transgenic expression cassette is also understood as meaning those constructs where the nucleic acid sequence encoding a yeast G3PDH is placed behind an endogenous plant promoter in such a way that the latter brings about the expression of the yeast G3PDH.

Promoters which are preferably introduced into the transgenic expression cassettes are those which are operable in a plant organism or a tissue, organ, part, cell or propagation material of same. Promoters which are operable in plant organisms is understood as meaning any promoter which is capable of governing the expression of genes, in particular foreign genes, in plants or plant parts, plant cells, plant tissues or plant cultures. In this context, expression may be, for example, constitutive, inducible or development-dependent.

The following are preferred:

a) Constitutive promoters

"Constitutive" promoters refers to those promoters which ensure expression in a large number of, preferably all, tissues over a substantial period of plant development, preferably at all times during plant development (Benfey et al.(1989) EMBO J 8:2195-2202). A plant promoter or promoter originating from a plant virus is especially preferably used. The promoter of the CaMV (cauliflower mosaic virus) 35S transcript (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221- 228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202) are especially preferred. Another suitable constitutive promoter is the Rubisco small subunit (SSU) promoter (US 4,962,028), the leguminB promoter (GenBank Acc. No. X03677), the promoter of the nopalin synthase from Agrobacterium, the TR dual promoter, the OCS (octopine synthase) promoter from Agrobacterium, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits, the promoter of the Arabidopsis thaliana nitrilase-1 gene (GenBank Acc. No.: U38846, nucleotides 3862 to 5325 or else 5342) or the promoter of a proline-rich protein from wheat (WO 91/13991), and further promoters of genes whose constitutive expression in plants is known to the skilled worker. The CaMV 35S promoter and the Arabidopsis thaliana nitrilase-1 promoter are particularly preferred.

b) Tissue-specific promoters

Furthermore preferred are promoters with specificities for seeds, such as, for example, the phaseolin promoter (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53), the promoter of the 2S albumin gene (Joseffson LG et al. (1987) J Biol Chem 262:12196- 12201), the legumine promoter (Shirsat A et al. (1989) Mol Gen Genet 215(2):326-331), the USP (unknown seed protein) promoter (Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67), the napin gene promoter (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), the promoter of the sucrose binding proteins (WO 00/26388) or the legumin B4 promoter (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 121-128; Bäumlein et al. (1992) Plant Journal

2(2):233-9; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), the Arabidopsis oleosin promoter (WO 98/45461), and the Brassica Bce4 promoter (WO 91/13980).

Further suitable seed-specific promoters are those of the gene encoding high-molecular weight glutenin (HMWG), gliadin, branching enyzme, ADP glucose pyrophosphatase (AGPase) or starch synthase. Promoters which are furthermore preferred are those which permit a seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. The promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promoters described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, the glutelin gene, the zein gene, the casirin gene or the secalin gene) can advantageously be employed.

c) Chemically inducible promoters

The expression cassettes may also contain a chemically inducible promoter (review article: Gatz et al. (1997) Annu Rev Plant Physiol Plant Mol Biol 48:89-108), by means of which the expression of the exogenous gene in the plant can be controlled at a particular point in time. Such promoters such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), a salicylic acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic acid-inducible promoter EP 0 335 528) or an ethanol-cyclohexanone-inducible promoter (WO 93/21334) can likewise be used. Also suitable is the promoter of the glutathione-S transferase isoform II gene (GST-II-27), which can be activated by exogenously applied safeners such as, for example, N,N-diallyl-2,2-dichloroacetamide (WO 93/01294) and which is operable in a large number of tissues of both monocots and dicots.

Particularly preferred are constitutive promoters, very particularly preferred seed-specific promoters, in particular the napin promoter and the USP promoter.

In addition, further promoters which make possible expression in further plant tissues or in other organisms such as, for example, E.coli bacteria, may be linked operably with the nucleic acid

sequence to be expressed. Suitable plant promoters are, in principle, all of the above-described promoters.

The nucleic acid sequences present in the transgenic expression cassettes according to the invention or transgenic vectors can be linked operably with further genetic control sequences besides a promoter. The term genetic control sequences is to be understood in the broad sense and refers to all those sequences which have an effect on the establishment or the function of the expression cassette according to the invention. Genetic control sequences modify, for example, transcription and translation in prokaryotic or eukaryotic organisms. The transgenic expression cassettes according to the invention preferably encompass a plant-specific promoter 5'-upstream of the nucleic acid sequence to be expressed recombinantly in each case and, as additional genetic control sequence, a terminator sequence 3'-downstream, and, if appropriate, further customary regulatory elements, in each case linked operably with the nucleic acid sequence to be expressed recombinantly.

Genetic control sequences also encompass further promoters, promoter elements or minimal promoters capable of modifying the expression-controlling properties. Thus, genetic control sequences can, for example, bring about tissue-specific expression which is additionally dependent on certain stress factors. Such elements are, for example, described for water stress, abscisic acid (Lam E and Chua NH, J Biol Chem 1991; 266(26): 17131 -17135) and thermal stress (Schoffl F et al. (1989) Mol Gen Genetics 217(2-3):246-53).

Further advantageous control sequences are, for example, in the Gram-positive promoters amy and SPO2, and in the yeast or fungal promotors ADC1, MFa, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH.

In principle all natural promoters with their regulatory sequences like those mentioned above may be used for the method according to the invention. In addition, synthetic promoters may also be used advantageously.

Genetic control sequences further also encompass the 5'-untranslated regions, introns or nonencoding 3'-region of genes, such as, for example, the actin-1 intron, or the Adh1-S intron 1, 2 and 6 (for general reference, see: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994)). It has been demonstrated that these may play a significant role in regulating gene expression. Thus, it has been demonstrated that 5'-untranslated sequences can enhance the

transient expression of heterologous genes. Translation enhancers which may be mentioned by way of example are the tobacco mosaic virus 5′ leader sequence (Gallie et al. (1987) Nucl Acids Res 15:8693-8711) and the like. They may furthermore promote tissue specificity (Rouster J et al. (1998) Plant J 15:435-440).

The transient expression cassette can advantageously contain one or more of what are known as enhancer sequences in operable linkage with the promoter, and these make possible an increased recombinant expression of the nucleic acid sequence. Additional advantageous sequences such as further regulatory elements or terminators may also be inserted at the 3′ end of the nucleic acid sequences to be expressed recombinantly. One or more copies of the nucleic acid sequences to be expressed recombinanly may be present in the gene construct.

Polyadenylation signals which are suitable as control sequences are plant polyadenylation signals, preferably those which correspond essentially to Agrobacterium tumefaciens T-DNA polyadenylation signals, in particular those of gene 3 of the T-DNA (octopine synthase) of the Ti plasmid pTiACHS (Gielen et al. (1984) EMBO J 3:835 et seq.) or functional equivalents thereof. Examples of particularly suitable terminator sequences are the OCS (octopin synthase) terminator and the NOS (nopaline synthase) terminator.

Control sequences are furthermore understood as those which make possible homologous recombination or insertion into the genome of a host organism, or removal from the genome. In the case of homologous recombination, for example, the coding sequence of the specific endogenous gene can be exchanged in a directed fashion for a sequence encoding a dsRNA. Methods such as the cre/lox technology permit the tissue-specific, possibly inducible, removal of the expression cassette from the genome of the host organism (Sauer B (1998) Methods. 14(4):381-92). Here, certain flanking sequences are added to the target gene (lox sequences), and these make possible removal by means of cre recombinase at a later point in time.

A recombinant expression cassette and the recombinant vectors derived from it may comprise further functional elements. The term functional element is to be understood in the broad sense and refers to all those elements which have an effect on generation, replication or function of the expression cassettes, vectors or transgenic organisms according to the invention.

Examples which may be mentioned, but not by way of limitation, are:

a) Selection markers which confer resistance to a metabolism inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456), antibiotics or biocides, preferably herbicides, such as, for example, kanamycin, G 418, bleomycin, hygromycin, or phosphinothricin and the like. Particularly preferred selection markers are those which confer resistance to herbicides. The following may be mentioned by way of example: DNA sequences which encode phosphinothricin acetyltransferases (PAT) and which inactivate glutamine synthase inhibitors (bar and pat gene), 5-enolpyruvylshikimate-3-phosphate synthase genes (EPSP synthase genes), which confer resistance to Glyphosate® (N-(phosphonomethyl)glycine), the gox gene, which encodes Glyphosate®-degrading enzyme (Glyphosate oxidoreductase), the deh gene (encoding a dehalogenase which inactivates dalapon), sulfonylurea- and imidazolinone-inactivating acetolactate synthases, and bxn genes which encode nitrilase enzymes which degrade bromoxynil, the aasa gene, which confers resistance to the antibiotic apectinomycin, the streptomycin phosphotransferase (SPT) gene, which permits resistance to streptomycin, the neomycin phosphotransferase (NPTII) gene, which confers resistance to kanamycin or geneticidin, the hygromycin phosphotransferase (HPT) gene, which confers resistance to hygromycin, the acetolactate synthase gene (ALS), which confers resistance to sulfonylurea herbicides (for example mutated ALS variants with, for example, the S4 and/or Hra mutation).

b) Reporter genes which encode readily quantifiable proteins and which allow the transformation efficacy or the expression site or time to be assessed via their color or enzyme activity. Very particularly preferred in this context are reporter proteins (Schenborn E, Groskreutz D. Mol Biotechnol. 1999; 13(1):29-44) such as the "green fluorescence protein" (GFP) (Sheen et al.(1995) Plant Journal 8(5):777-784), chloramphenicol transferase, a luciferase (Ow et al. (1986) Science 234:856-859), the aequorin gene (Prasher et al. (1985) Biochem Biophys Res Commun 126(3):1259-1268), ß-galactosidase, with ß-glucuronidase being very particularly preferred (Jefferson et al. (1987) EMBO J 6:3901-3907).

c) Replication origins which allow replication of the expression cassettes or vectors according to the invention in, for example, E.coli. Examples which may be mentioned are ORI

(origin of DNA replication), the pBR322 ori or the P15A ori (Sambrook et al.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

d) Elements which are required for agrobacterium-mediated plant transformation such as, for example, the right or left border of the T-DNA, or the vir region.

To select cells which have successfully undergone homologous recombination or else cells which have succesfully been transformed, it is generally required additionally to introduce a selectable marker which confers resistance to a biocide (for example a herbicide), a metabolism inhibitor such as 2-deoxyglucose-6-phosphate (WO 98/45456) or an antibiotic to the cells which have successfully undergone recombination. The selection marker permits the selection of the transformed cells from untransformed cells (McCormick et al. (1986) Plant Cell Reports 5:81-84).

In addition, said recombinant expression cassette or vectors may comprise further nucleic acid sequences which do not encode a yeast G3PDH and whose recombinant expression leads to a further increase in fatty acid biosynthesis (as a consequence of proOIL). By way of example, but not by limitation, this proOIL nucleic acid sequence which is additionally expressed recombinantly can be selected from among nucleic acids encoding acetyl-CoA carboxylase (ACCase), glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidate acyltransferase (LPAT), diacylglycerol acyltransferase (DAGAT) and phospholipid:diacylglycerol acyltransferase (PDAT). Such sequences are known to the skilled worker and are readily accessible from databases or suitable cDNA libraries of the respective plants.

An expression cassette according to the invention can advantageously be introduced into an organism or cells, tissues, organs, parts or seeds thereof (preferably into plants or plant cells, tissues, organs, parts or seeds) by using vectors in which the recombinant expression cassettes are present. The invention therefore furthermore relates to said recombinant vectors which encompass a recombinant expression cassette for a yeast G3PDH.

For example, vectors may be plasmids, cosmids, phages, viruses or else agrobacteria. The expression cassette can be introduced into the vector (preferably a plasmid vector) via a suitable restriction cleavage site. The resulting vector is first introduced into E.coli. Correctly transformed E.coli are

selected, grown, and the recombinant vector is obtained with methods known to the skilled worker. Restriction analysis and sequencing may be used for verifying the cloning step. Preferred vectors are those which make possible stable integration of the expression cassette into the host genome.

The invention furthermore relates to transgenic plant organisms or tissues, organs, parts, cells or propagation material thereof which comprise a yeast G3PDH as defined above, a transgenic expression cassette for a yeast G3PDH or a transgenic vector encompassing such an expression cassette.

Such a transgenic plant organism is generated, for example, by means of transformation or transfection by means of the corresponding proteins or nucleic acids. The generation of a transformed organism (or a transformed cell or tissue) requires introducing the DNA in question (for example the expression vector), RNA or protein into the host cell in question. A multiplicity of methods is available for this procedure, which is termed transformation (or transduction or transfection) (Keown et al. (1990) Methods in Enzymology 185:527-537). Thus, the DNA or RNA can be introduced for example directly by microinjection or by bombardment with DNA-coated microparticles. The cell may also be permeabilized chemically, for example with polyethylene glycol, so that the DNA may reach the cell by diffusion. The DNA can also be carried out by protoplast fusion with other DNA-comprising units such as minicells, cells, lysosomes or liposomes. Electroporation is a further suitable method for introducing DNA; here, the cells are permeabilized reversibly by an electrical pulse. Soaking plant parts in DNA solutions, and pollen or pollen tube transformation, are also possible. Such methods have been described (for example in Bilang et al. (1991) Gene 100:247-250; Scheid et al. (1991) Mol Gen Genet 228:104-112; Guerche et al. (1987) Plant Science 52:111-116; Neuhause et al. (1987) Theor Appl Genet 75:30-36; Klein et al. (1987) Nature 327:70-73; Howell et al. (1980) Science 208:1265; Horsch et al.(1985) Science 227:1229-1231; DeBlock et al. (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press Inc. (1989)).

In plants, the methods which have been described for transforming and regenerating plants from plant tissues or plant cells are exploited for transient or stable transformation. Suitable methods are, in particular, protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic method with

the gene gun, what is known as the particle bombardment method, electroporation, the incubation of dry embryos in DNA-containing solution, and microinjection.

In addition to these "direct" transformation techniques, transformation may also be effected by bacterial infection by means of Agrobacterium tumefaciens or Agrobacterium rhizogenes and the transfer of corresponding recombinant Ti plasmids or Ri plasmids by or by infection with transgenic plant viruses. Agrobacterium-mediated transformation is best suited to cells of dicotyledonous plants. The methods are described, for example, in Horsch RB et al. (1985) Science 225: 1229f).

When agrobacteria are used, the expression cassette is to be integrated into specific plasmids, either into a shuttle vector or into a binary vector. If a Ti or Ri plasmid is to be used for the transformation, at least the right border, but in most cases the right and left border, of the Ti or Ri plasmid T-DNA is linked to the expression cassette to be introduced as flanking region.

Binary vectors are preferably used. Binary vectors are capable of replication both in E.coli and in Agrobacterium. As a rule, they contain a selection marker gene and a linker or polylinker flanked by the right and left T-DNA border sequence. They can be transformed directly into Agrobacterium (Holsters et al. (1978) Mol Gen Genet 163:181-187). The selection marker gene, which is, for example, the nptII gene, which confers resistance to kanamycin, permits a selection of transformed agrobacteria. The agrobacterium which acts as host organism in this case should already contain a plasmid with the vir region. The latter is required for transferring the T-DNA to the plant cells. An agrobacterium transformed in this way can be used for transforming plant cells. The use of T-DNA for the transformation of plant cells has been studied intensively and described (EP 120 516; Hoekema, In: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasserdam, Chapter V; An et al. (1985) EMBO J 4:277-287). Various binary vectors, some of which are commercially available, such as, for example, pBI101.2 or pBIN19 (Clontech Laboratories, Inc. USA), are known.

Further promoters which are suitable for expression in plants have been described (Rogers et al. (1987) Meth in Enzymol 153:253-277; Schardl et al. (1987) Gene 61:1-11; Berger et al. (1989) Proc Natl Acad Sci USA 86:8402-8406).

Direct transformation techniques are suitable for any organism and cell type. In cases where DNA or RNA are injected or electroporated into plant cells, the plasmid used need not meet any particular requirements. Simple plasmids such as those from the pUC series may be used. If intact plants are to be regenerated from the transformed cells, it is necessary for an additional selectable marker gene to be present on the plasmid.

Stably transformed cells, i.e. those which contain the inserted DNA integrated into the DNA of the host cell, can be selected from untransformed cells when a selectable marker is part of the inserted DNA. By way of example, any gene which is capable of conferring resistance to antibiotics or herbicides (such as kanamycin, G 418, bleomycin, hygromycin or phosphinothricin and the like) is capable of acting as marker (see above). Transformed cells which express such a marker gene are capable of surviving in the presence of concentrations of such an antibiotic or herbicide which kill an untransformed wild type. Examples are mentioned above and preferably comprise the bar gene, which confers resistance to the herbicide phosphinothricin (Rathore KS et al. (1993) Plant Mol Biol 21(5):871-884), the nptII gene, which confers resistance to kanamycin, the hpt gene, which confers resistance to hygromycin, or the EPSP gene, which confers resistance to the herbicide Glyphosate. The selection marker permits selection of transformed cells from untransformed cells (McCormick et al. (1986) Plant Cell Reports 5:81-84). The plants obtained can be bred and hybridized in the customary manner. Two or more generations should be grown in order to ensure that the genomic integration is stable and hereditary.

The above-described methods are described, for example, in Jenes B et al.(1993) Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by SD Kung and R Wu, Academic Press, pp.128-143, and in Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225). The construct to be expressed is preferably cloned into a vector which is suitable for transforming Agrobacterium tumefaciens, for example pBin19 (Bevan et al. (1984) Nucl Acids Res 12:8711f).

Once a transformed plant cell has been generated, an intact plant can be obtained using methods known to the skilled worker. For example, callus cultures are used as starting material. The development of shoot and root can be induced in this as yet undifferentiated cell biomass in the known fashion. The plantlets obtained can be planted out and used for breeding.

The skilled worker is familiar with such methods for regenerating plant parts and intact plants from plant cells. Methods which can be used for this purpose are, for example, those described by Fennell et al. (1992) Plant Cell Rep. 11: 567-570; Stoeger et al (1995) Plant Cell Rep. 14:273-278; Jahne et al. (1994) Theor Appl Genet 89:525-533.

"Transgenic", for example in the case of a yeast G3PDH, refers to a nucleic acid sequence, an expression cassette or a vector comprising said G3PDH nucleic acid sequence or to an organism transformed with said nucleic acid sequence, expression cassette or vector all those constructs established by recombinant methods in which either

a) the nucleic acid sequence encoding a yeast G3PDH or

b) a genetic control sequence, for example a promoter which is functional in plant organisms, which is linked operably with said nucleic acid sequence under a), or

c) (a) and (b)

are not in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to be, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. Natural genetic environment refers to the natural chromosomal locus in the source organism or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least to some extent. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, particularly preferably at least 1000 bp, very particularly preferably at least 5000 bp. A naturally occurring expression cassette, for example the naturally occurring combination of the promoter of a gene encoding for a yeast G3PDH with the corresponding yeast G3PDH gene, becomes a transgenic expression cassette when the latter is modified by non-natural, synthetic ("artificial") methods such as, for example, a mutagenization. Such methods are described (US 5,565,350; WO 00/15815; see also above).

Host or starting organisms which are preferred as transgenic organisms are, above all, plants in accordance with the above definition. Included for the purposes of the invention are all genera and species of higher and lower plants of the Plant

Kingdom, in particular plants which are used for obtaining oils, such as, for example, oilseed rape, sunflower, sesame, safflower, olive tree, soya, maize, wheat and nut species. Furthermore included are the mature plants, seed, shoots and seedlings, and parts, propagation material and cultures, for example cell cultures, derived therefrom. Mature plants refers to plants at any desired developmental stage beyond the seedling stage. Seedling refers to a young, immature plant at an early developmental stage.

The transgenic organisms can be generated with the above-described methods for the transformation or transfection of organisms.

The invention furthermore relates to the use of the transgenic organisms according to the invention and to the cells, cell cultures, parts - such as, for example, in the case of transgenic plant organisms roots, leaves and the like - and transgenic propagation material such as seeds or fruits which are derived therefrom for the production of foodstuffs or feedstuffs, pharmaceuticals or fine chemicals, in particular oils, fats, fatty acids or derivatives of these.

Besides influencing the oil content, the transgenic expression of a yeast G3PDH in plants may mediate yet further advantageous effects such as, for example, an increased stress resistance to, for example, osmotic stress. Via increased glycerol levels, the yeast G3PDH confers protection against this type of stress, with glycerol acting as osmoprotective substance. Such osmotic stress occurs for example in saline soils and water and is an increasing problem in agriculture. Increased stress tolerance makes it possible, for example, to use areas in which conventional arable plants are not capable of thriving for agricultural usage.

Furthermore, recombinant expression of the yeast G3PDH can influence the NADH level and thus the redox balance in the plant organism. Stress such as, for example, drought, high or low temperatures, UV light and the like can lead to increased NADH levels and to an increased formation of reactive oxygen (RO). Transgenic expression of the yeast G3PDH can break down excessive NADH, which accumulates under said stress conditions, and thus stabilize the redox balance and alleviate the effects of the stress.

Sequences

1. SEQ ID NO: 1
Nucleic acid sequence encoding Saccharomyces cerevisiae G3PDH (Gpd1p)

2. SEQ ID NO: 2
Protein sequence encoding Saccharomyces cerevisiae G3PDH (Gpd1p)

3. SEQ ID NO: 3
Nucleic acid sequence encoding Saccharomyces cerevisiae G3PDH (Gpd2p)

4. SEQ ID NO: 4
Protein sequence encoding Saccharomyces cerevisiae G3PDH (Gpd2p)

5. SEQ ID NO: 5
Protein sequence encoding Saccharomyces cerevisiae G3PDH (Gpd2p) with second alternative start codon

6. SEQ ID NO: 6
Nucleic acid sequence encoding Schizosaccharomyces pombe G3PDH

7. SEQ ID NO: 7
Protein sequence encoding Schizosaccharomyces pombe G3PDHD

8. SEQ ID NO: 8
Nucleic acid sequence encoding Schizosaccharomyces pombe G3PDH

9. SEQ ID NO: 9
Protein sequence encoding Schizosaccharomyces pombe G3PDH

10. SEQ ID NO: 10
Nucleic acid sequence encoding Yarrowinia lipolytica G3PDH

11. SEQ ID NO: 11
Protein sequence encoding Yarrowinia lipolytica G3PDH

12. SEQ ID NO: 12
Protein sequence encoding Yarrowinia lipolytica G3PDH, with second alternative start codon

13. SEQ ID NO: 13
Nucleic acid sequence encoding Zygosaccharomyces rouxii G3PDH

14. SEQ ID NO: 14
Protein sequence encoding Zygosaccharomyces rouxii G3PDH

15. SEQ ID NO: 15
Nucleic acid sequence encoding Zygosaccharomyces rouxii G3PDH

16. SEQ ID NO: 16
Protein sequence encoding Zygosaccharomyces rouxii G3PDH

17. SEQ ID NO: 16
Expression vector based on pSUN-USP for S.cerevisiae G3PDH (Gpd1p; 1017 - 2190 bp insert)

18. SEQ ID NO: 18 Oligonucleotide primer ONP1
5'-ACTAGTATGTCTGCTGCTGCTGATAG-3'

19. SEQ ID NO: 19 Oligonucleotide primer ONP2
5'-CTCGAGATCTTCATGTAGATCTAATT-3'

20. SEQ ID NO: 20 Oligonucleotide primer ONP3
5'-GCGGCCGCCATGTCTGCTGCTGCTGATAG-3'

21. SEQ ID NO: 21 Oligonucleotide primer ONP4
5'-GCGGCCGCATCTTCATGTAGATCTAATT-3'

22-35: SEQ ID NP 22 to 35: Sequence motifs for yeast G3PDHs; possible sequence variations are given. The variations of an individual motif may occur in each case alone, but also in the different combinations with each other.

36. SEQ ID NO: 36
Expression vector pGPTV-gpd1 based on pGPTV-napin for S.cerevisiae G3PDH (Gpd1p; gdp1 insert of 11962-13137 bp; nos terminator: 13154-13408; napin promoter: 10807-11951).

37. SEQ ID NO: 37
Nucleic acid sequence encoding Emericella nidulans G3PDH

38. SEQ ID NO: 38
Amino acid encoding Emericella nidulans G3PDH

39. SEQ ID NO: 39
Nucleic acid sequence encoding Debaryomyces hansenii G3PDH (partial)

40. SEQ ID NO: 40
Amino acid encoding Debaryomyces hansenii G3PDH (partial)

Figures

Fig. 1: Oil content in transgenic GPD1p lines

Measurement of the TAG content in T2 seeds of transgenic Arabidopsis lines with the Saccharomyces cerevisiae Gpd1p gene (G2 to G30). The content in corresponding untransformed plants (wild-type plants; W1 to W10) has been determined for comparison. 8 Arabidopsis lines with a significantly increased oil content were identified. The error deviation stated is the result of 3 independent measurements in each case.

Fig. 2: Determination of the oil content in seeds of the T3 generation

The data shown are the oil content (in mg lipid per g dry matter (DM)) of individual Arabidopsis lines. Each column represents the mean of 6 individual plants per independent line. Each plant was analysed in triplicate. The error bars denote the standard deviation over all values. The control plants are identified by "col". The numerical values of the individual data are additionally shown in the following table (the control was set as 100% oil content):

Lines	Oil content (mg/g)	STD	Rel. increase in %
col	278.1	12.2	100
#11	304.6	18.3	110
#12	301.4	19.0	108
#13	275.2	89.7	99
#21	323.2	77.0	116
#24	268.9	15.1	97
#25	293.6	23.0	106
#27	285.6	18.4	103
#41	316.1	19.1	114
#53	260.3	16.4	94
#67	292.0	13.8	105
#71	244.1	11.6	88
#82	295.6	16.8	106

Lines with a statistically significantly increased lipid content (lines #11, #21, #41 and #67) are presented as a black bar.

Fig. 3: Determination of the G3PDH activity in the control ("col") and the gdp1-transformed plants.

The G3PDG activity of the individual lines was determined as decribed in Example 8 and is shown in nmol G3P per minute per g of fresh weight (FW).

	G3PDH Activity	STD
col	6.68337432	0.71785229
#11	11.8958635	1.67941604
#12	9.14226124	2.25411878
#13	8.8210768	2.19519777
#21	9.88435444	1.04798566
#24	5.89378595	1.26005769
#25	5.14179348	1.22845409
#27	6.77303725	3.22220935
#41	20.8325636	5.42018531
#53	7.45794947	2.25573816
#67	12.7670015	0.74678353
#71	9.04748534	1.59829185
#82	9.37260033	2.1356558

Lines with a statistically significantly increased G3PDH activity (lines #11, #21, #41 and #67) are presented as a black bar. It can be seen that an increased G3PDG activity correlates with an increased lipid content.

Examples

General methods:

Unless otherwise specified, all chemicals were from Fluka (Buchs), Merck (Darmstadt), Roth (Karlsruhe), Serva (Heidelberg) and Sigma (Deisenhofen). Restriction enzymes, DNA-modifying enzymes and molecular biological kits were from Amersham-Pharmacia (Freiburg), Biometra (Göttingen), Roche (Mannheim), New England Biolabs (Schwalbach), Novagen (Madison, Wisconsin, USA), Perkin-Elmer (Weiterstadt), Qiagen (Hilden), Stratagen (Amsterdam, Netherlands), Invitrogen (Karlsruhe) and Ambion (Cambridgeshire, United Kingdom). The reagents used were employed in accordance with the manufacturer's instructions.

For example, oligonucleotides can be synthesized chemically in the known manner using the phosphoamidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). The cloning steps carried out for the purposes of the present invention such as, for example, restriction cleavages, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linking DNA fragments, transformation of E. coli cells, bacterial cultures, multiplication of phages and sequence analysis of recombinant DNA, are carried out as decribed by Sambrook et al. (1989) Cold Spring Harbor Laboratory Press; ISBN 0-87969-309-6. Recombinant DNA molecules were sequenced using an ABI laser fluorescence DNA sequencer following the method of Sanger (Sanger et al. (1977) Proc Natl Acad Sci USA 74:5463-5467).

Example 1: General methods

The plant Arabidopsis thaliana belongs to the higher plants (flowering plants). This plant is closely related to other plant species from the Cruciferae family such as, for example, Brassica napus, but also to other families of dicotyledonous plants. Owing to the high degree of homology of its DNA sequences or its polypeptide sequences, Arabidopsis thaliana can be employed as model plant for other plant species.

a) Culture of Arabidopsis plants

The plants are grown either on Murashige-Skoog medium supplemented with 0.5 % sucrose (Ogas et al. (1997) Science 277:91-94) or in soil (Focks & Benning (1998) Plant Physiol 118:91-101). To achieve uniform germination and flowering times, the seeds are first placed on medium or scattered on the soil and then stratified for two days at 4°C. After flowering, the pods are labeled. According to the labels, pods aged 6 to 20 days post-anthesis are then harvested.

Example 2: Cloning the yeast Gpd1 gene

Genomic DNA from Saccharomyces cerevisiae strain S288C (Mat alpha SUC2 mal mel gal2 CUP1 flo1 flo8-1; Invitrogen, Karlsruhe, Germany) was isolated following the protocol described hereinbelow:

A 100 ml culture was grown at 30°C to an optical density of 1.0. 60 ml of the culture were spun down for 3 minutes at 3000 x g. The pellet was resuspended in 6 ml of twice-distilled H_2O and the suspension was divided between 1.5 ml containers and spun down,

and the supernatant was discarded. The pellets were resuspended in 200 µl of solution A, 200 µl phenol/chloroform (1:1) and 0.3 g of glass beads by vortexing and then lysed. After addition of 200 µl of TE buffer, pH 8.0, the lysates were spun for 5 minutes. The supernatant was subjected to ethanol precipitation with 1 ml of ethanol. After the precipitation, the resulting pellet was dissolved in 400 µl of TE buffer pH 8.0 + 30 µg/ml RNase A. Following incubation for 5 minutes at 37°C, 18 µl 3 M sodium acetate solution pH 4.8 and 1 ml of ethanol were added, and the precipitated DNA was pelleted by spinning. The DNA pellet was dissolved in 25 µl of twice-distilled H_2O. The concentration of the genomic DNA was determined by its absorption at 260 nm.

Solution A:
2 % Trition-X100
1 % SDS
0.1 M NaCl
0.01 M Tris-HCl pH 8.0
0.001 M EDTA

To clone the Gpd1 gene, the yeast DNA which has been isolated was employed in a PCR reaction with the oligonucleotide primers ONP1 and ONP2.

ONP1: 5'-ACTAGTATGTCTGCTGCTGCTGATAG-3' (SEQ ID NO: 18)
ONP2: 5'-CTCGAGATCTTCATGTAGATCTAATT-3' (SEQ ID NO: 19)

Composition of the PCR reaction (50 µl):

5.00 µl 5 µg genomic yeast-DNA
5.00 µl 10x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl each primer (10 pmol/uL)
0.50 µl Advantage polymerase

The Advantage polymerase employed was from Clontech.

PCR-Program:
Initial denaturation for 2 min at 95°C, then 35 cycles of 45 sec at 95°C, 45 sec at 55°C and 2 min at 72°C. Final extension for 5 min at 72°C.

The PCR products were cloned into the vector pCR2.1-TOPO (Invitrogen) following the manufacturer's instructions, resulting in the vector pCR2.1-gpd1, and the sequence was verified by sequencing.

Cloning into the agro transformation vector pGPTV involved incubating 0.5 µg of the vector pCR2.1-gpd1 with the restriction enzyme XhoI (New England Biolabs) for 2 hours and subsequent incubation for 15 minutes with Klenow fragment (New England Biolabs). After incubation for 2 hours with SpeI, the DNA fragments were separated by gel electrophoresis. The 1185 bp segment of the gpd1 sequence next to the vector (3.9 kb) was excized from the gel, purified with the "Gel Purification" kit from Qiagen following the manufacturer's instructions and eluted with 50 µl of elution buffer. 0.1 µg of the vector pGPTV was first digested for 1 hour with the restriction enzyme SacI and then incubated for 15 minutes with Klenow fragment (New England Biolabs). 10 µl of the eluate of the gpd1 fragments and 10 ng of the treated pGPTV vector were ligated overnight at 16°C (T4 ligase, New England Biolabs). The ligation products were then transformed into TOP10 cells (Stratagene) following the manufacturer's instructions and suitably selected, resulting in the vector pGPTV-gpd1. Positive clones are verified by sequencing and PCR using the primers ONP1 and ONP2.

To generate the vector pSUN-USP-gpd1, a PCR was carried out with the vector pCR2.1-gpd1 using the primers ONP3 and ONP4.

ONP3: 5'-GCGGCCGCCATGTCTGCTGCTGCTGATAG-3' (SEQ ID NO: 20)
ONP4: 5'-GCGGCCGCATCTTCATGTAGATCTAATT-3' (SEQ ID NO: 21)
Composition of the PCR reaction (50 µl):

5 ng DNA plasmid pCR2.1-gpd1
5.00 µl 10x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl each primer (10 pmol/uL)
0.50 µl Advantage polymerase

The Advantage polymerase employed was from Clontech.

PCR-Program:
Initial denaturation for 2 min at 95°C, then 35 cycles of 45 sec at 95°C, 45 sec at 55°C and 2 min at 72°C. Final extension for 5 min at 72°C.

The 1190 bp PCR product was digested for 24 hours with the restriction enzyme NotI. The vector pSUN-USP was digested for 2 hours with NotI and then incubated for 15 minutes with alkaline phosphatase (New England Biolabs). 100 ng of the pretreated gpd1 fragment and 10 ng of the treated vector pGPTV were ligated overnight at 16°C (T4 Ligase from New England Biolabs). The ligation products were then transformed into TOP10 cells

(Stratagene) following the manufacturer's instructions and suitably selected, resulting in the vector pSUN-USP-gpd1. Positive clones are verified by sequencing and PCR using the primers ONP3 and ONP4.

Example 3: Plasmids for the transformation of plants

Binary vectors such as pBinAR can be used for the transformation of plants (Höfgen und Willmitzer (1990) Plant Science 66: 221-230). The binary vectors can be constructed by ligating the cDNA into T-DNA in sense and antisense orientation. 5' of the cDNA, a plant promoter activates the transcription of the cDNA. A polyadenylation sequence is located 3' of the cDNA.

Tissue-specific expression can be achieved using a tissue-specific promoter. For example, seed-specific expression can be achieved by cloning in the napin or the LeB4- or the USP promoter 5' of the cDNA. Any other seed-specific promoter element can also be used. The CaMV 35S promoter can be used for constitutive expression in the whole plant.

A further example of binary vectors is the vector pSUN-USP and pGPTV-napin, into which the fragment of Example 2 was cloned. The vector pSUN-USP contains the USP promoter and the OCS terminator. The vector pGPTV-napin contains a truncated version of the napin promoter, and the nos terminator.

The fragments of Example 2 were cloned into the multiple cloning site of the vector pSUN-USP and pGPTV-napin respectively, to make possible the seed-specific expression of the gdp1 gene. The corresponding construct pSUN-USP-gpd1 is described with the SEQ ID NO: 17, and the construct of G3PDH in pGPTV-napin (pGPTV-gpd1) by SEQ ID NO: 36.

Example 4: Transformation of Agrobacterium

Agrobacterium-mediated plant transformation can be carried out for example using the Agrobacterium tumefaciens strains GV3101 (pMP90) (Koncz und Schell (1986) Mol Gen Genet 204: 383-396) or LBA4404 (Clontech). Standard transformation techniques may be used for the transformation (Deblaere et al.(1984) Nucl Acids Res 13:4777-4788).

Example 5: Transformation of plants

Agrobacterium-mediated plant transformation can be effected using standard transformation and regeneration techniques (Gelvin SB, Schilperoort R, Plant Molecular Biology Manual, 2nd ed., Dordrecht: Kluwer Academic Publ., 1995, in Sect., Ringbuch Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick BR, Thompson JE, Methods in Plant Molecular Biology and Biotechnology, Boca Raton: CRC Press, 1993, 360 pp., ISBN 0-8493-5164-2).

The transformation of Arabidopsis thaliana by means of Agrobacterium was carried out by the method of Bechthold et al., 1993 (C.R. Acad. Sci. Ser. III Sci. Vie., 316, 1194-1199).

For example, oilseed rape can be transformed by cotyledon or hypocotyl transformation (Moloney et al.(1989) Plant Cell Report 8:238-242; De Block et al.(1989) Plant Physiol 91: 694-701). The use of antibiotics for the selection of agrobacteria and plants depends on the binary vector used for the transformation and the agrobacterial strain. The selection of oilseed rape is usually carried out using kanamycin as selectable plant marker.

Agrobacterium-mediated gene transfer into linseed (Linum usitatissimum) can be carried out for example using a technique described by Mlynarova et al. (1994) Plant Cell Report 13:282-285. Soya can be transformed for example using a technique described in EP-A-0 0424 047 (Pioneer Hi-Bred International) or in EP-A-0 0397 687, US 5,376,543, US 5,169,770 (University of Toledo).

The transformation of plants using particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbonate fiber technique is described, for example, by Freeling and Walbot "The Maize Handbook" (1993) ISBN 3-540-97826-7, Springer Verlag New York).

Example 6: Studying the expression of a recombinant gene product in a transformed organism

The activity of a recombinant gene product in the transformed host organism was measured at the transcription and/or translation level.

A suitable method for determining the level of transcription of the gene (which indicates the amount of RNA available for translating the gene product) is to carry out a Northern blot as described hereinbelow (for reference see Ausubel et al. (1988)

Current Protocols in Molecular Biology, Wiley: New York, or the above examples section), where a primer which is designed such that it binds to the gene of interest is labeled with a detectable label (usually a radiolabel or chemiluminescent label) so that, when the total RNA of a culture of the organism is extracted, separated on a gel, transferred to a stable matrix and incubated with this probe, binding and the extent of binding of the probe indicates the presence and the amount of mRNA for this gene. This information indicates the degree of transcription of the transformed gene. Cellular total RNA can be prepared from cells, tissues or organs using several methods, all of which are known in the art, for example the method Bormann, E.R., et al. (1992) Mol. Microbiol. 6:317-326.

Northern hybridization:

To carry out the RNA hybridization, 20 µg of total RNA or 1 µg of poly(A)+ RNA were separated by means of gel electrophoresis in 1.25% strength agarose gels using formaldehyde and following the method described by Amasino (1986, Anal. Biochem. 152, 304), transferred to positively charged nylon membranes (Hybond N+, Amersham, Brunswick) by capillary force using 10 x SSC, immobilized by UV light and prehybridized for 3 hours at 68°C using hybridization buffer (10% dextran sulfate w/v, 1 M NaCl, 1 % SDS, 100 mg herring sperm DNA). The DNA probe was labeled with the Highprime DNA labeling kit (Roche, Mannheim, Germany) during the prehybridization step, using alpha-^{32}P-dCTP (Amersham Pharmacia, Brunswick, Germany). Hybridization was carried out overnight at 68°C after addition of the labeled DNA probe in the same buffer. The wash steps were carried out twice for 15 minutes using 2 X SSC and twice for 30 minutes using 1 X SSC, 1% SDS, at 68°C. The sealed filters were exposed at -70°C for a period of 1 to 14 days.

To study the presence or the relative amount of protein translated from this mRNA, standard techniques such as a Western blot may be employed (see, for example, Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York). In this method, the cellular total proteins are extracted, separated by means of gel electrophoresis, transferred to a matrix like nitrocellulose and incubated with a probe such as an antibody which binds specifically to the desired protein. This probe is usually provided with a chemiluminescent or colorimetric label which can be detected readily. The presence and the amount of the label observed indicates the presence and the amount of the desired mutated protein which is present in the cell.

Example 7: Analysis of the effect of the recombinant proteins on the production of the desired product

The effect of genetic modification in plants, fungi, algae, ciliates or on the production of a desired compound (such as a fatty acid) can be determined by growing the modified microorganisms or the modified plant under suitable conditions (as described above) and examining the medium and/or the cellular components for increased production of the desired product (i.e. lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various staining methods, enzymatic and microbiological methods, and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullmann, Encyclopedia of Industrial Chemistry, vol. A2, pp. 89-90 and pp. 443-613, VCH: Weinheim (1985); Fallon A et al. (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al. (1993) Biotechnology, vol. 3, chapter III: "Product recovery and purification", pp. 469-714, VCH: Weinheim; Belter PA et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy JF und Cabral JMS (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz JA and Henry JD (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, vol. B3; chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned methods, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, and Browse et al. (1986) Analytic Biochemistry 152:141-145. Qualitative and quantitative lipid or fatty acid analysis is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

In addition to measuring the end product of the fermentation, it is also possible to analyze other components of the metabolic pathways which are used for producing the desired compound, such as intermediates and secondary products, in order to determine the overall efficacy of the production of the compound. The

analytical methods encompass measurements of the nutrient quantities in the medium (for example sugars, carbohydrates, nitrogen sources, phosphate and other ions), measurements of the biomass compositions and of the growth, analysis of the production of customary metabolites of biosynthetic pathways, and measurements of gases produced during fermentation. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, ed., IRL Press, pp. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAME, fatty acid methyl esters; GC-MS, gas-liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

Unambiguous proof for the presence of fatty acid products can be obtained by analyzing recombinant organisms by analytical standard methods: GC, GC-MS or TLC, as described variously by Christie and the references cited therein (1997, in: Advances on Lipid Methodology, fourth edition: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren [gas-chromatographic/mass-spectrometric methods], Lipide 33:343-353).

The material to be analyzed can be disrupted by sonication, milling in the glass mill, liquid nitrogen and milling or other applicable methods. After disruption, the material must be centrifuged. The sediment is resuspended in distilled water, heated for 10 minutes at 100°C, cooled on ice and recentrifuged, followed by extraction in 0.5 M sulfuric acid in methanol with 2% dimethoxypropane for 1 hour at 90°C, which gives hydrolyzed oil and lipid compounds, which give transmethylated lipids. These fatty acid methyl esters are extracted in petroleum ether and finally subjected to GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 mm, 0.32 mm) at a temperature gradient of between 170°C and 240°C for 20 minutes and for 5 minutes at 240°C. The identity of the fatty acid methyl esters obtained must be defined using standards which are available from commercial sources (i.e. Sigma).

The following protocol was used for the quantitative oil analysis of the Arabidopsis plants transformed with the Gpd1 gene:

Lipid extraction from the seeds is carried out by the method of Bligh & Dyer (1959) Can J Biochem Physiol 37:911. To this end, 5 mg of Arabidopsis seeds are weighed into 1.2 ml Qiagen microtubes

(Qiagen, Hilden) using a Sartorius (Göttingen) microbalance. The seed material is homogenized with 500 µl chloroform/methanol (2:1; contains mono-C17-glycerol from Sigma as internal standard) in an MM300 Retsch mill from Retsch (Haan) and incubated for 20 minutes at RT. The phases were separated after addition of 500 µl 50 mM potassium phosphate buffer pH 7.5. 50 µl are removed from the organic phase, diluted with 1500 µl of chloroform, and 5 µl are applied to Chromarods SIII capillaries from Iatroscan (SKS, Bechenheim). After application of the samples, they are separated in a first step for 15 mins in a thin-layer chamber saturated with 6:2:2 chloroform: methanol: toluene. After the time has elapsed, the capillaries are dried for 4 minutes at room temperature and then placed for 22 minutes into a thin-layer chamber saturated with 7:3 n-hexane:diethyl ether. After a further drying step for 4 minutes at room temperature, the samples are analyzed in an Iatroscan MK-5 (SKS, Bechenheim) following the method of Fraser & Taggart, 1988 J. Chromatogr. 439:404. The following parameters were set for the measurements: slice width 50 msec, threshold 20 mV, noise 30, skim ratio 0. The data were quantified with reference to the internal standard mono-C17-glycerol (Sigma) and a calibration curve established with tri-C17-glycerol (Sigma), using the program ChromStar (SKS, Beichenheim).

T2 seeds of several independent transgenic lines with the constructs pSUN-USP-gpd1 or pGPTV-gpd1 were analyzed to determine the oil contents quantitatively. Three independent extractions were carried out with the seed pools of each line, and the extracts were measured independently. The three independent measurements were used to calculate the mean and the standard deviation.

The result of the measurements for the lines with the construct pGPTV-gpd1 showed a significantly higher oil content in several (10) transgenic lines (Fig. 1) compared to the measurements of 10 wild-type plants. Similar oil contents are measured for the construct pSUN-USP-gpd1 (not shown).

The average oil content of the above lines is 34.86 ± 1.56%, while the average of the wild-type plants is 27.75 ± 2.64%. This corresponds to an absolute increase in the oil content of 7.1% (relative: 25.6%).

To verify the heritability of the gdp1 effect (increased oil content), T2 seeds from the lines with increased oil contents and from lines with unchanged oil contents were planted. In each case 6 plants per line were planted out and the seeds were analyzed

for oil content and enzyme activity. The oil content was determined by the methodology described above. The data obtained are shown in Fig. 2. Col-0 and C24 Arabidopsis ecotypes act as controls. C24 is an ecotype which is distinguished by a higher oil content than Col-0. It was possible to characterize lines whose oil contents exceeds that of Col-0. The heritability of the increased oil content as the effect of the expression of the gdp1 genes was thus demonstrated.

Example 8: Determination of glycerol-3-phosphate dehydrogenase activity

A further aim was the demonstration of the direct effect of the enzyme in the transgenic plants, in addition to the increased oil content. To determine the glycerol-3-phosphate dehydrogenase activity, two Arabidopsis seed pods were harvested per plant and extracted by the method of Geigenberger and Stitt ((1993) Planta 189:329-339). To this end, the pods were ground in a mortar under liquid nitrogen and taken up in 200 µl 50 mM HEPES pH 7.4 5 mM $MgCl_2$, 1 mM EDTA, 1mM EGTA, 5mM DTT, 0.1 % (w/w) of bovine serum albumin, 2mM benzamidine, 2mM amino-n-caproic acid, 0.5 mM phenylmethylsulphonyl, 0.1% Triton X-100 and 10% (w/w) glycerol and spun down for 5 minutes, and the supernatant was divided into aliquots. The production of G3P (glycerol-3-phosphate) from the substrates DHAP (dihydroxyacetone phosphate) and NADH was measured to determine the G3PDH activity. To this end, the oxidation of NADH was monitored at 340 nm.

The reaction mixture for the activity determination contained 50 mM HEPES pH 7.4, 4 mM DHAP, 0.2 mM NADH and 10 µl of the extraction mix in final volume of 100 µl. After incubation for 30 minutes at room temperature, the reaction was stopped by heating (20 min, 95□C). In the control reaction, the reaction was stopped immediately by heating.

Glycerol-3-phosphate "cycling assay": 10 µl of the reaction mixture were added to 45 µl of a solution comprising 200 mM Tricin, $MgCl_2$ 5mM (pH 8.5) and heated (20 min, 95□C) to destroy remaining DHAP. The supernatant was transferred into a 96-well microtiter plate, treated with 45 µl of a mixture comprising 2 units G3Pox, 130 units catalase, 0.4 unit G3PDH and 0.12 µmol NADH. The reaciton was carried out at 30□C and the resulting absorption monitored at 340 nm in an Anthos htII microplate reader. Reaction rates were calculated on the basis of the

decrease in absorption in (mOD*min-1) using the Biolise software (gibon Y et al. (2002) Plant J 30(2):221-235).

The enzyme activity in the transgenic lines #11, #21, #41 and #67 is significantly higher than in control plants (Fig. 3). The plants with increased oil contents correlate with plants with increased enzyme activites. It was thus demonstrated that the increased oil content can be attributed to the increased conversion of DHAP into G3P, the precursor of oil synthesis.

SEQUENCE LISTING

<110> BASF Plant Science GmbH

<120> Method for increasing the oil content in plants

<130> NAE 2166/2002

<140>

<141>

<160> 40

<170> PatentIn Ver. 2.1

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<223> coding for G3PDH

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gct ggt aga aag aga agt tcc tct tct gtt tct ttg aag gct gcc gaa   96
Ala Gly Arg Lys Arg Ser Ser Ser Ser Val Ser Leu Lys Ala Ala Glu
             20                  25                  30

aag cct ttc aag gtt act gtg att gga tct ggt aac tgg ggt act act   144
Lys Pro Phe Lys Val Thr Val Ile Gly Ser Gly Asn Trp Gly Thr Thr
         35                  40                  45

att gcc aag gtg gtt gcc gaa aat tgt aag gga tac cca gaa gtt ttc   192
Ile Ala Lys Val Val Ala Glu Asn Cys Lys Gly Tyr Pro Glu Val Phe
     50                  55                  60

gct cca ata gta caa atg tgg gtg ttc gaa gaa gag atc aat ggt gaa   240
Ala Pro Ile Val Gln Met Trp Val Phe Glu Glu Glu Ile Asn Gly Glu
 65                  70                  75                  80

aaa ttg act gaa atc ata aat act aga cat caa aac gtg aaa tac ttg   288
Lys Leu Thr Glu Ile Ile Asn Thr Arg His Gln Asn Val Lys Tyr Leu
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cct ggc atc act cta ccc gac aat ttg gtt gct aat cca gac ttg att   336
Pro Gly Ile Thr Leu Pro Asp Asn Leu Val Ala Asn Pro Asp Leu Ile
            100                 105                 110

gat tca gtc aag gat gtc gac atc atc gtt ttc aac att cca cat caa   384
Asp Ser Val Lys Asp Val Asp Ile Ile Val Phe Asn Ile Pro His Gln
        115                 120                 125

ttt ttg ccc cgt atc tgt agc caa ttg aaa ggt cat gtt gat tca cac   432
Phe Leu Pro Arg Ile Cys Ser Gln Leu Lys Gly His Val Asp Ser His
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gtc caa ttg cta tcc tct tac atc act gag gaa cta ggt att caa tgt   528
Val Gln Leu Leu Ser Ser Tyr Ile Thr Glu Glu Leu Gly Ile Gln Cys
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ggt gct cta tct ggt gct aac att gcc acc gaa gtc gct caa gaa cac   576
Gly Ala Leu Ser Gly Ala Asn Ile Ala Thr Glu Val Ala Gln Glu His
            180                 185                 190

tgg tct gaa aca aca gtt gct tac cac att cca aag gat ttc aga ggc   624
Trp Ser Glu Thr Thr Val Ala Tyr His Ile Pro Lys Asp Phe Arg Gly
        195                 200                 205

gag ggc aag gac gtc gac cat aag gtt cta aag gcc ttg ttc cac aga   672
Glu Gly Lys Asp Val Asp His Lys Val Leu Lys Ala Leu Phe His Arg
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cct tac ttc cac gtt agt gtc atc gaa gat gtt gct ggt atc tcc atc   720
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225                 230                 235                 240

tgt ggt gct ttg aag aac gtt gtt gcc tta ggt tgt ggt ttc gtc gaa   768
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ggt cta ggc tgg ggt aac aac gct tct gct gcc atc caa aga gtc ggt   816
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ttg ggt gag atc atc aga ttc ggt caa atg ttt ttc cca gaa tct aga   864
Leu Gly Glu Ile Ile Arg Phe Gly Gln Met Phe Phe Pro Glu Ser Arg
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gaa gaa aca tac tac caa gag tct gct ggt gtt gct gat ttg atc acc   912
Glu Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile Thr
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acc tgc gct ggt ggt aga aac gtc aag gtt gct agg cta atg gct act   960
Thr Cys Ala Gly Gly Arg Asn Val Lys Val Ala Arg Leu Met Ala Thr
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tct ggt aag gac gcc tgg gaa tgt gaa aag gag ttg ttg aat ggc caa  1008
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aca tgt ggc tct gtc gaa gac ttc cca tta ttt gaa gcc gta tac caa  1104
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atc gtt tac aac aac tac cca atg aag aac ctg ccg gac atg att gaa  1152
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Ala Pro Ile Val Gln Met Trp Val Phe Glu Glu Glu Ile Asn Gly Glu
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Asp Ser Val Lys Asp Val Asp Ile Ile Val Phe Asn Ile Pro His Gln
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Phe Leu Pro Arg Ile Cys Ser Gln Leu Lys Gly His Val Asp Ser His
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Val Arg Ala Ile Ser Cys Leu Lys Gly Phe Glu Val Gly Ala Lys Gly
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Val Gln Leu Leu Ser Ser Tyr Ile Thr Glu Glu Leu Gly Ile Gln Cys
165 170 175
Gly Ala Leu Ser Gly Ala Asn Ile Ala Thr Glu Val Ala Gln Glu His
180 185 190
Trp Ser Glu Thr Thr Val Ala Tyr His Ile Pro Lys Asp Phe Arg Gly
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Glu Gly Lys Asp Val Asp His Lys Val Leu Lys Ala Leu Phe His Arg
210 215 220
Pro Tyr Phe His Val Ser Val Ile Glu Asp Val Ala Gly Ile Ser Ile
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Cys Gly Ala Leu Lys Asn Val Val Ala Leu Gly Cys Gly Phe Val Glu
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305                 310                 315                 320

Ser Gly Lys Asp Ala Trp Glu Cys Glu Lys Glu Leu Leu Asn Gly Gln
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Ser Ala Gln Gly Leu Ile Thr Cys Lys Glu Val His Glu Trp Leu Glu
            340                 345                 350

Thr Cys Gly Ser Val Glu Asp Phe Pro Leu Phe Glu Ala Val Tyr Gln
        355                 360                 365

Ile Val Tyr Asn Asn Tyr Pro Met Lys Asn Leu Pro Asp Met Ile Glu
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Glu Leu Asp Leu His Glu Asp
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cat ccg gtg tta tat act cgt cgt gca tat aaa att ttg cct tca aga   96
His Pro Val Leu Tyr Thr Arg Arg Ala Tyr Lys Ile Leu Pro Ser Arg
             20                  25                  30

tct act ttc cta aga aga tca tta tta caa aca caa ctg cac tca aag   144
Ser Thr Phe Leu Arg Arg Ser Leu Leu Gln Thr Gln Leu His Ser Lys
         35                  40                  45

atg act gct cat act aat atc aaa cag cac aaa cac tgt cat gag gac   192
Met Thr Ala His Thr Asn Ile Lys Gln His Lys His Cys His Glu Asp
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cat cct atc aga aga tcg gac tct gcc gtg tca att gta cat ttg aaa   240
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 65                  70                  75                  80
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                85                  90                  95
acc atc gcc aaa gtc att gcg gaa aac aca gaa ttg cat tcc cat atc   336
Thr Ile Ala Lys Val Ile Ala Glu Asn Thr Glu Leu His Ser His Ile
            100                 105                 110
ttc gag cca gag gtg aga atg tgg gtt ttt gat gaa aag atc ggc gac   384
Phe Glu Pro Glu Val Arg Met Trp Val Phe Asp Glu Lys Ile Gly Asp
        115                 120                 125
gaa aat ctg acg gat atc ata aat aca aga cac cag aac gtt aaa tat   432
Glu Asn Leu Thr Asp Ile Ile Asn Thr Arg His Gln Asn Val Lys Tyr
    130                 135                 140
cta ccc aat att gac ctg ccc cat aat cta gtg gcc gat cct gat ctt   480
Leu Pro Asn Ile Asp Leu Pro His Asn Leu Val Ala Asp Pro Asp Leu
145                 150                 155                 160
tta cac tcc atc aag ggt gct gac atc ctt gtt ttc aac atc cct cat   528
Leu His Ser Ile Lys Gly Ala Asp Ile Leu Val Phe Asn Ile Pro His
                165                 170                 175
caa ttt tta cca aac ata gtc aaa caa ttg caa ggc cac gtg gcc cct   576
Gln Phe Leu Pro Asn Ile Val Lys Gln Leu Gln Gly His Val Ala Pro
            180                 185                 190
cat gta agg gcc atc tcg tgt cta aaa ggg ttc gag ttg ggc tcc aag   624
His Val Arg Ala Ile Ser Cys Leu Lys Gly Phe Glu Leu Gly Ser Lys
        195                 200                 205
ggt gtg caa ttg cta tcc tcc tat gtt act gat gag tta gga atc caa   672
Gly Val Gln Leu Leu Ser Ser Tyr Val Thr Asp Glu Leu Gly Ile Gln
    210                 215                 220
tgt ggc gca cta tct ggt gca aac ttg gca ccg gaa gtg gcc aag gag   720
Cys Gly Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu
225                 230                 235                 240
cat tgg tcc gaa acc acc gtg gct tac caa cta cca aag gat tat caa   768
His Trp Ser Glu Thr Thr Val Ala Tyr Gln Leu Pro Lys Asp Tyr Gln
                245                 250                 255
ggt gat ggc aag gat gta gat cat aag att ttg aaa ttg ctg ttc cac   816
Gly Asp Gly Lys Asp Val Asp His Lys Ile Leu Lys Leu Leu Phe His
            260                 265                 270
aga cct tac ttc cac gtc aat gtc atc gat gat gtt gct ggt ata tcc   864
Arg Pro Tyr Phe His Val Asn Val Ile Asp Asp Val Ala Gly Ile Ser
        275                 280                 285
att gcc ggt gcc ttg aag aac gtc gtg gca ctt gca tgt ggt ttc gta   912
Ile Ala Gly Ala Leu Lys Asn Val Val Ala Leu Ala Cys Gly Phe Val
    290                 295                 300
gaa ggt atg gga tgg ggt aac aat gcc tcc gca gcc att caa agg ctg   960
Glu Gly Met Gly Trp Gly Asn Asn Ala Ser Ala Ala Ile Gln Arg Leu
305                 310                 315                 320
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aaa gtc gag acc tac tat caa gaa tcc gct ggt gtt gca gat ctg atc   1056
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acc acc tgc tca ggc ggt aga aac gtc aag gtt gcc aca tac atg gcc   1104
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        355                 360                 365

aag acc ggt aag tca gcc ttg gaa gca gaa aag gaa ttg ctt aac ggt   1152
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cca gat agt cta caa caa cgt ccg cat gga aga cct acc gga gat gat   1296
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His Pro Ile Arg Arg Ser Asp Ser Ala Val Ser Ile Val His Leu Lys
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Arg Ala Pro Phe Lys Val Thr Val Ile Gly Ser Gly Asn Trp Gly Thr
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Thr Ile Ala Lys Val Ile Ala Glu Asn Thr Glu Leu His Ser His Ile
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Phe Glu Pro Glu Val Arg Met Trp Val Phe Asp Glu Lys Ile Gly Asp
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Glu Asn Leu Thr Asp Ile Ile Asn Thr Arg His Gln Asn Val Lys Tyr
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Gln Phe Leu Pro Asn Ile Val Lys Gln Leu Gln Gly His Val Ala Pro
180 185 190
His Val Arg Ala Ile Ser Cys Leu Lys Gly Phe Glu Leu Gly Ser Lys
195 200 205
Gly Val Gln Leu Leu Ser Ser Tyr Val Thr Asp Glu Leu Gly Ile Gln
210 215 220
Cys Gly Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu
225 230 235 240
His Trp Ser Glu Thr Thr Val Ala Tyr Gln Leu Pro Lys Asp Tyr Gln
245 250 255
Gly Asp Gly Lys Asp Val Asp His Lys Ile Leu Lys Leu Leu Phe His
260 265 270
Arg Pro Tyr Phe His Val Asn Val Ile Asp Asp Val Ala Gly Ile Ser
275 280 285
Ile Ala Gly Ala Leu Lys Asn Val Val Ala Leu Ala Cys Gly Phe Val
290 295 300
Glu Gly Met Gly Trp Gly Asn Asn Ala Ser Ala Ala Ile Gln Arg Leu
305 310 315 320
Gly Leu Gly Glu Ile Ile Lys Phe Gly Arg Met Phe Phe Pro Glu Ser
325 330 335
Lys Val Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile
340 345 350
Thr Thr Cys Ser Gly Gly Arg Asn Val Lys Val Ala Thr Tyr Met Ala
355 360 365
Lys Thr Gly Lys Ser Ala Leu Glu Ala Glu Lys Glu Leu Leu Asn Gly
370 375 380
Gln Ser Ala Gln Gly Ile Ile Thr Cys Arg Glu Val His Glu Trp Leu
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Glu Asn Leu Thr Asp Ile Ile Asn Thr Arg His Gln Asn Val Lys Tyr
                 85                  90                  95
Leu Pro Asn Ile Asp Leu Pro His Asn Leu Val Ala Asp Pro Asp Leu
            100                 105                 110
Leu His Ser Ile Lys Gly Ala Asp Ile Leu Val Phe Asn Ile Pro His
        115                 120                 125
Gln Phe Leu Pro Asn Ile Val Lys Gln Leu Gln Gly His Val Ala Pro
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His Val Arg Ala Ile Ser Cys Leu Lys Gly Phe Glu Leu Gly Ser Lys
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Cys Gly Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu
            180                 185                 190
His Trp Ser Glu Thr Thr Val Ala Tyr Gln Leu Pro Lys Asp Tyr Gln
        195                 200                 205
Gly Asp Gly Lys Asp Val Asp His Lys Ile Leu Lys Leu Leu Phe His
    210                 215                 220
Arg Pro Tyr Phe His Val Asn Val Ile Asp Asp Val Ala Gly Ile Ser
225                 230                 235                 240
Ile Ala Gly Ala Leu Lys Asn Val Val Ala Leu Ala Cys Gly Phe Val
                245                 250                 255
Glu Gly Met Gly Trp Gly Asn Asn Ala Ser Ala Ala Ile Gln Arg Leu
            260                 265                 270
Gly Leu Gly Glu Ile Ile Lys Phe Gly Arg Met Phe Phe Pro Glu Ser
        275                 280                 285
Lys Val Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile
    290                 295                 300
Thr Thr Cys Ser Gly Gly Arg Asn Val Lys Val Ala Thr Tyr Met Ala
305                 310                 315                 320
Lys Thr Gly Lys Ser Ala Leu Glu Ala Glu Lys Glu Leu Leu Asn Gly
                325                 330                 335
Gln Ser Ala Gln Gly Ile Ile Thr Cys Arg Glu Val His Glu Trp Leu
            340                 345                 350
Gln Thr Cys Glu Leu Thr Gln Glu Phe Pro Ile Ile Arg Gly Ser Leu
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caa aaa tct ttt tca cct aaa ctt att tct gtt ggt atc atc gga tca   96
Gln Lys Ser Phe Ser Pro Lys Leu Ile Ser Val Gly Ile Ile Gly Ser
            20                  25                  30

gga aat tgg gga acc gct att gct aaa ata tgt ggt gaa aat gcc aag  144
Gly Asn Trp Gly Thr Ala Ile Ala Lys Ile Cys Gly Glu Asn Ala Lys
        35                  40                  45

gct cat cct gat att ttc cat cct caa gta cac atg tgg atg tat gaa  192
Ala His Pro Asp Ile Phe His Pro Gln Val His Met Trp Met Tyr Glu
    50                  55                  60

gag aag att caa cat gag gga aaa gag tgc aat ctc acg gaa gtt ttt  240
Glu Lys Ile Gln His Glu Gly Lys Glu Cys Asn Leu Thr Glu Val Phe
65                  70                  75                  80

aac act act cat gaa aac gtt aaa tat ctc aaa ggt atc aaa tgc cct  288
Asn Thr Thr His Glu Asn Val Lys Tyr Leu Lys Gly Ile Lys Cys Pro
                85                  90                  95

tct aac gtc ttc gca aac ccg gac att cgt gat gta ggt tca cgt agc  336
Ser Asn Val Phe Ala Asn Pro Asp Ile Arg Asp Val Gly Ser Arg Ser
            100                 105                 110

gac att ctg gta tgg gtt ctc cct cac cag ttc gtt gtg cgt att tgc  384
Asp Ile Leu Val Trp Val Leu Pro His Gln Phe Val Val Arg Ile Cys
        115                 120                 125

aat caa ttg aag gga tgc cta aag aag gat gct gtt gca att tca tgc  432
Asn Gln Leu Lys Gly Cys Leu Lys Lys Asp Ala Val Ala Ile Ser Cys
    130                 135                 140

atc aaa ggt gta tct gtc acc aag gac cgt gtt cgc ctc ttt tct gat  480
Ile Lys Gly Val Ser Val Thr Lys Asp Arg Val Arg Leu Phe Ser Asp
145                 150                 155                 160

att atc gaa gaa aac acg gga atg tat tgt ggc gtt ctc tct ggc gcc  528
Ile Ile Glu Glu Asn Thr Gly Met Tyr Cys Gly Val Leu Ser Gly Ala
                165                 170                 175

aac att gcc agc gaa gtt gct caa gag aag ttt tgc gaa act act atc  576
Asn Ile Ala Ser Glu Val Ala Gln Glu Lys Phe Cys Glu Thr Thr Ile
            180                 185                 190
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gga tat ttg cct aat agt tct gtt aat ccc cgc tat act cct aag act   624
Gly Tyr Leu Pro Asn Ser Ser Val Asn Pro Arg Tyr Thr Pro Lys Thr
        195                 200                 205

atc caa gct ttg ttt aac cgt ccc tac ttc cgt gtc aac att gtt gag   672
Ile Gln Ala Leu Phe Asn Arg Pro Tyr Phe Arg Val Asn Ile Val Glu
    210                 215                 220

gat gtt cct ggt gtt gct ttg ggc ggt gca ctc aag aat atc gtc gct   720
Asp Val Pro Gly Val Ala Leu Gly Gly Ala Leu Lys Asn Ile Val Ala
225                 230                 235                 240

gtc gct gcc ggt att att gat gga ctt gaa ttg gga gat aat acc aaa   768
Val Ala Ala Gly Ile Ile Asp Gly Leu Glu Leu Gly Asp Asn Thr Lys
                245                 250                 255

tct gct gtt atg cgc att ggc ctt ctg gaa atg cag aaa ttc ggc agg   816
Ser Ala Val Met Arg Ile Gly Leu Leu Glu Met Gln Lys Phe Gly Arg
            260                 265                 270

atg ttt ttc gat tgt aag cct ctt act atg agc gag gaa tct tgt ggc   864
Met Phe Phe Asp Cys Lys Pro Leu Thr Met Ser Glu Glu Ser Cys Gly
        275                 280                 285

ata gcc gat tta att aca act tgc tta ggc ggc cgt aac cac aaa tgc   912
Ile Ala Asp Leu Ile Thr Thr Cys Leu Gly Gly Arg Asn His Lys Cys
    290                 295                 300

gct gtg gca ttt gtc aag aca gga aag ccc atg cat gtt gtt gaa caa   960
Ala Val Ala Phe Val Lys Thr Gly Lys Pro Met His Val Val Glu Gln
305                 310                 315                 320

gaa ctt ctt gat ggt cag aag ttg caa ggt gca gct acc gcg aag gag   1008
Glu Leu Leu Asp Gly Gln Lys Leu Gln Gly Ala Ala Thr Ala Lys Glu
                325                 330                 335

gtt tat gag ttc ctt gat aac cag aat aag gta agc gaa ttc cca ttg   1056
Val Tyr Glu Phe Leu Asp Asn Gln Asn Lys Val Ser Glu Phe Pro Leu
            340                 345                 350

ttt aca gct gtt tat cgc att gtt tat gag gga ctt cca cct aat aag   1104
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        355                 360                 365

ctt ctg gag gct att taa                                          1122
Leu Leu Glu Ala Ile
    370
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<210> 7
<211> 373
<212> PRT
<213> Schizosaccharomyces pombe

<400> 7

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Met Thr Val Ala Ala Leu Asn Lys Leu Ser Ala Leu Ser Gly Ser Ile
  1               5                  10                  15

Gln Lys Ser Phe Ser Pro Lys Leu Ile Ser Val Gly Ile Ile Gly Ser
             20                  25                  30
```

Gly Asn Trp Gly Thr Ala Ile Ala Lys Ile Cys Gly Glu Asn Ala Lys
35 40 45
Ala His Pro Asp Ile Phe His Pro Gln Val His Met Trp Met Tyr Glu
50 55 60
Glu Lys Ile Gln His Glu Gly Lys Glu Cys Asn Leu Thr Glu Val Phe
65 70 75 80
Asn Thr Thr His Glu Asn Val Lys Tyr Leu Lys Gly Ile Lys Cys Pro
85 90 95
Ser Asn Val Phe Ala Asn Pro Asp Ile Arg Asp Val Gly Ser Arg Ser
100 105 110
Asp Ile Leu Val Trp Val Leu Pro His Gln Phe Val Val Arg Ile Cys
115 120 125
Asn Gln Leu Lys Gly Cys Leu Lys Lys Asp Ala Val Ala Ile Ser Cys
130 135 140
Ile Lys Gly Val Ser Val Thr Lys Asp Arg Val Arg Leu Phe Ser Asp
145 150 155 160
Ile Ile Glu Glu Asn Thr Gly Met Tyr Cys Gly Val Leu Ser Gly Ala
165 170 175
Asn Ile Ala Ser Glu Val Ala Gln Glu Lys Phe Cys Glu Thr Thr Ile
180 185 190
Gly Tyr Leu Pro Asn Ser Ser Val Asn Pro Arg Tyr Thr Pro Lys Thr
195 200 205
Ile Gln Ala Leu Phe Asn Arg Pro Tyr Phe Arg Val Asn Ile Val Glu
210 215 220
Asp Val Pro Gly Val Ala Leu Gly Gly Ala Leu Lys Asn Ile Val Ala
225 230 235 240
Val Ala Ala Gly Ile Ile Asp Gly Leu Glu Leu Gly Asp Asn Thr Lys
245 250 255
Ser Ala Val Met Arg Ile Gly Leu Leu Glu Met Gln Lys Phe Gly Arg
260 265 270
Met Phe Phe Asp Cys Lys Pro Leu Thr Met Ser Glu Glu Ser Cys Gly
275 280 285
Ile Ala Asp Leu Ile Thr Thr Cys Leu Gly Gly Arg Asn His Lys Cys
290 295 300
Ala Val Ala Phe Val Lys Thr Gly Lys Pro Met His Val Val Glu Gln
305 310 315 320
Glu Leu Leu Asp Gly Gln Lys Leu Gln Gly Ala Ala Thr Ala Lys Glu
325 330 335
Val Tyr Glu Phe Leu Asp Asn Gln Asn Lys Val Ser Glu Phe Pro Leu
340 345 350
Phe Thr Ala Val Tyr Arg Ile Val Tyr Glu Gly Leu Pro Pro Asn Lys
355 360 365
Leu Leu Glu Ala Ile
370

<210> 8
<211> 1155
<212> DNA
<213> Schizosaccharomyces pombe

<220>
<221> CDS
<222> (1)..(1152)
<223> coding for G3PDH

<400> 8

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atg tct gga tat ggt caa caa ggt gtt tct gct gcc aac atc gac agc   48
Met Ser Gly Tyr Gly Gln Gln Gly Val Ser Ala Ala Asn Ile Asp Ser
  1               5                  10                  15

atc cgc ccc aag aaa cgt ttg tca att ggt gta gtt ggc tcc ggt aac   96
Ile Arg Pro Lys Lys Arg Leu Ser Ile Gly Val Val Gly Ser Gly Asn
             20                  25                  30

tgg ggt act gcc att gcc aag att tgc ggt gaa aat gcc cgt gcc cac   144
Trp Gly Thr Ala Ile Ala Lys Ile Cys Gly Glu Asn Ala Arg Ala His
         35                  40                  45

ggt cac cat ttc aga ggt aag ggg cgc atg tgg gtc ttt gag gag gag   192
Gly His His Phe Arg Gly Lys Gly Arg Met Trp Val Phe Glu Glu Glu
     50                  55                  60

att gag tac aag ggt gag aag aga aag ctc acc gaa gta ttc aac gaa   240
Ile Glu Tyr Lys Gly Glu Lys Arg Lys Leu Thr Glu Val Phe Asn Glu
 65                  70                  75                  80

gct cac gag aat gtc aaa tac tta ccc ggc atc gaa tgc cct ccc aac   288
Ala His Glu Asn Val Lys Tyr Leu Pro Gly Ile Glu Cys Pro Pro Asn
                 85                  90                  95

gtt att gcc gtc ccc gat gtt cgt gag gtc gct aga cgt gcc gac atc   336
Val Ile Ala Val Pro Asp Val Arg Glu Val Ala Arg Arg Ala Asp Ile
            100                 105                 110

ctt gtc ttt gtc gtt cct cat caa ttt att gaa cgc gtt tgg cac caa   384
Leu Val Phe Val Val Pro His Gln Phe Ile Glu Arg Val Trp His Gln
        115                 120                 125

atg gtc ggt ctc att cgc cct ggt gcc gtt ggt att tcc tgt atc aag   432
Met Val Gly Leu Ile Arg Pro Gly Ala Val Gly Ile Ser Cys Ile Lys
    130                 135                 140

ggt gtt gct gtc agc aag gaa ggc tcg ctt tac tct gag gtt atc agc   480
Gly Val Ala Val Ser Lys Glu Gly Ser Leu Tyr Ser Glu Val Ile Ser
145                 150                 155                 160

gag aaa ctc ggt att tac tgt ggt gtt ctt tct ggt gct aac gtt gca   528
Glu Lys Leu Gly Ile Tyr Cys Gly Val Leu Ser Gly Ala Asn Val Ala
                165                 170                 175
```

```
aac gaa gtt gcc cgt gag caa ttc tgt gag act act att ggt ttc aac   576
Asn Glu Val Ala Arg Glu Gln Phe Cys Glu Thr Thr Ile Gly Phe Asn
            180                 185                 190

cct cct aat gaa gtt gat atc cct cgc gag caa atc gcc gcc gtc tct   624
Pro Pro Asn Glu Val Asp Ile Pro Arg Glu Gln Ile Ala Ala Val Ser
        195                 200                 205

gat cgc cct tac ttc tca gtt gtc tcc gtt gac gac gtt gcc ggt gtc   672
Asp Arg Pro Tyr Phe Ser Val Val Ser Val Asp Asp Val Ala Gly Val
    210                 215                 220

gcc ttg ggt ggt gct ttg aag aac gta gtt gcc atg gcc gtt ggt ttc   720
Ala Leu Gly Gly Ala Leu Lys Asn Val Val Ala Met Ala Val Gly Phe
225                 230                 235                 240

gct gat ggt ttg gaa tgg ggc ggt aat acc aag gcc gct att atg cgt   768
Ala Asp Gly Leu Glu Trp Gly Gly Asn Thr Lys Ala Ala Ile Met Arg
                245                 250                 255

cgt ggt ttg ttg gag atg caa aag ttt gct act acc ttc ttc gac tct   816
Arg Gly Leu Leu Glu Met Gln Lys Phe Ala Thr Thr Phe Phe Asp Ser
            260                 265                 270

gat cct cgt acc atg gtt gag caa tct tgc ggt atc gct gac ttg gtc   864
Asp Pro Arg Thr Met Val Glu Gln Ser Cys Gly Ile Ala Asp Leu Val
        275                 280                 285

act tct tgt ttg ggt ggc cgt aac aat cgt tgt gct gaa gca ttt gtc   912
Thr Ser Cys Leu Gly Gly Arg Asn Asn Arg Cys Ala Glu Ala Phe Val
    290                 295                 300

aag act ggt aaa tct tta gag acg ctt gaa aaa gag ctc tta ggt ggt   960
Lys Thr Gly Lys Ser Leu Glu Thr Leu Glu Lys Glu Leu Leu Gly Gly
305                 310                 315                 320

caa ctt ctt caa gga gct gcc act tcc aag gat gtt cat gaa ttc ctt  1008
Gln Leu Leu Gln Gly Ala Ala Thr Ser Lys Asp Val His Glu Phe Leu
                325                 330                 335

ctc acc aag gat atg gtc aag gat ttc ccc ttg ttc act gcc gtt tat  1056
Leu Thr Lys Asp Met Val Lys Asp Phe Pro Leu Phe Thr Ala Val Tyr
            340                 345                 350

aac att tcc tat gaa gac atg gat ccc aag gat ttg atc atc gtc ctt  1104
Asn Ile Ser Tyr Glu Asp Met Asp Pro Lys Asp Leu Ile Ile Val Leu
        355                 360                 365

caa ccc ctt aag gag gac tct gag aac gag ggc ggt act gaa acc gag  1152
Gln Pro Leu Lys Glu Asp Ser Glu Asn Glu Gly Gly Thr Glu Thr Glu
    370                 375                 380

taa                                                             1155
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<210> 9
<211> 384
<212> PRT
<213> Schizosaccharomyces pombe

<400> 9

Met Ser Gly Tyr Gly Gln Gln Gly Val Ser Ala Ala Asn Ile Asp Ser
1 5 10 15

Ile Arg Pro Lys Lys Arg Leu Ser Ile Gly Val Val Gly Ser Gly Asn
20 25 30

Trp Gly Thr Ala Ile Ala Lys Ile Cys Gly Glu Asn Ala Arg Ala His
35 40 45

Gly His His Phe Arg Gly Lys Gly Arg Met Trp Val Phe Glu Glu Glu
50 55 60

Ile Glu Tyr Lys Gly Glu Lys Arg Lys Leu Thr Glu Val Phe Asn Glu
65 70 75 80

Ala His Glu Asn Val Lys Tyr Leu Pro Gly Ile Glu Cys Pro Pro Asn
85 90 95

Val Ile Ala Val Pro Asp Val Arg Glu Val Ala Arg Arg Ala Asp Ile
100 105 110

Leu Val Phe Val Val Pro His Gln Phe Ile Glu Arg Val Trp His Gln
115 120 125

Met Val Gly Leu Ile Arg Pro Gly Ala Val Gly Ile Ser Cys Ile Lys
130 135 140

Gly Val Ala Val Ser Lys Glu Gly Ser Leu Tyr Ser Glu Val Ile Ser
145 150 155 160

Glu Lys Leu Gly Ile Tyr Cys Gly Val Leu Ser Gly Ala Asn Val Ala
165 170 175

Asn Glu Val Ala Arg Glu Gln Phe Cys Glu Thr Thr Ile Gly Phe Asn
180 185 190

Pro Pro Asn Glu Val Asp Ile Pro Arg Glu Gln Ile Ala Ala Val Ser
195 200 205

Asp Arg Pro Tyr Phe Ser Val Val Ser Val Asp Asp Val Ala Gly Val
210 215 220

Ala Leu Gly Gly Ala Leu Lys Asn Val Val Ala Met Ala Val Gly Phe
225 230 235 240

Ala Asp Gly Leu Glu Trp Gly Gly Asn Thr Lys Ala Ala Ile Met Arg
245 250 255

Arg Gly Leu Leu Glu Met Gln Lys Phe Ala Thr Thr Phe Phe Asp Ser
260 265 270

Asp Pro Arg Thr Met Val Glu Gln Ser Cys Gly Ile Ala Asp Leu Val
275 280 285

Thr Ser Cys Leu Gly Gly Arg Asn Asn Arg Cys Ala Glu Ala Phe Val
290 295 300

Lys Thr Gly Lys Ser Leu Glu Thr Leu Glu Lys Glu Leu Leu Gly Gly
305 310 315 320

Gln Leu Leu Gln Gly Ala Ala Thr Ser Lys Asp Val His Glu Phe Leu
325 330 335

Leu Thr Lys Asp Met Val Lys Asp Phe Pro Leu Phe Thr Ala Val Tyr
340 345 350
Asn Ile Ser Tyr Glu Asp Met Asp Pro Lys Asp Leu Ile Ile Val Leu
355 360 365
Gln Pro Leu Lys Glu Asp Ser Glu Asn Glu Gly Gly Thr Glu Thr Glu
370 375 380

<210> 10
<211> 1197
<212> DNA
<213> Yarrowia lipolytica
<220>
<221> CDS
<222> (1)..(1194)
<223> coding for G3PDH
<220>
<221> CDS
<222> (40)..(1194)
<400> 10

atg agc gct cta ctt aga tcg tcc ctg cgt ttt aaa cac atg tcc gcc 48
Met Ser Ala Leu Leu Arg Ser Ser Leu Arg Phe Lys His Met Ser Ala
1 5 10 15
gtc aac cgt ctc aca caa cag ctt cga ctg ctg acc gcc tcc gcg cct 96
Val Asn Arg Leu Thr Gln Gln Leu Arg Leu Leu Thr Ala Ser Ala Pro
20 25 30
ctc agc gca gcc aac acc gcc ggc aag gct cct ttc aag gtc gcc gtt 144
Leu Ser Ala Ala Asn Thr Ala Gly Lys Ala Pro Phe Lys Val Ala Val
35 40 45
gtt ggt tct ggt aac tgg gga acc acc gtc gcc aag att gtc gcc gag 192
Val Gly Ser Gly Asn Trp Gly Thr Thr Val Ala Lys Ile Val Ala Glu
50 55 60
aac tgc act gct cac ccc gag ctc ttt gag ccc gag gtt cga gtc tgg 240
Asn Cys Thr Ala His Pro Glu Leu Phe Glu Pro Glu Val Arg Val Trp
65 70 75 80
gtt cga gaa gag aag gtc aac ggc aag aac ctg acc gac att ttc aac 288
Val Arg Glu Glu Lys Val Asn Gly Lys Asn Leu Thr Asp Ile Phe Asn
85 90 95
gct gag cac gag aac gtg cga tac ctc cct aaa atc aaa ctt cct cac 336
Ala Glu His Glu Asn Val Arg Tyr Leu Pro Lys Ile Lys Leu Pro His
100 105 110
aac ctg atc gcc gag ccg gat ctg ctc aag gcc gtc gag ggt gcc aac 384
Asn Leu Ile Ala Glu Pro Asp Leu Leu Lys Ala Val Glu Gly Ala Asn
115 120 125

```
atc atc gtc ttc aac ctg ccc cat cag ttc ctg gct ggt gtc tgc aag   432
Ile Ile Val Phe Asn Leu Pro His Gln Phe Leu Ala Gly Val Cys Lys
    130                 135                 140
cag ctc aag ggc cac gtc aac ccc aag gct aga gcc atc tcc tgc ctc   480
Gln Leu Lys Gly His Val Asn Pro Lys Ala Arg Ala Ile Ser Cys Leu
145                 150                 155                 160
aag ggt cta gat gtc acc ccc cag ggt gtt tac ctg ctc tcc gac gtt   528
Lys Gly Leu Asp Val Thr Pro Gln Gly Val Tyr Leu Leu Ser Asp Val
                165                 170                 175
atc gag aac gag acc ggt ctc cac tgc ggt gtt ctg tcc ggg gct aac   576
Ile Glu Asn Glu Thr Gly Leu His Cys Gly Val Leu Ser Gly Ala Asn
            180                 185                 190
ctc gcc acc gag atc gct ctg gag aag tac tcc gag act acc gtt gct   624
Leu Ala Thr Glu Ile Ala Leu Glu Lys Tyr Ser Glu Thr Thr Val Ala
        195                 200                 205
tac aac cga ccc aag gac ttc ttt ggc gag ggt gat gtg acc aac gat   672
Tyr Asn Arg Pro Lys Asp Phe Phe Gly Glu Gly Asp Val Thr Asn Asp
    210                 215                 220
gtg ctc aag gct ctg ttc cac cga ccc tac ttc cat gtg cga tgc gtt   720
Val Leu Lys Ala Leu Phe His Arg Pro Tyr Phe His Val Arg Cys Val
225                 230                 235                 240
cag gac gtc gcc ggt gtc tcc atc gga ggt gcc ctt aag aac gtt gtt   768
Gln Asp Val Ala Gly Val Ser Ile Gly Gly Ala Leu Lys Asn Val Val
                245                 250                 255
gcc ctt tgc gcc ggt ttc gtc gag ggc aag aac tgg gga gac aac gcc   816
Ala Leu Cys Ala Gly Phe Val Glu Gly Lys Asn Trp Gly Asp Asn Ala
            260                 265                 270
aag gcc gca att atg cga cga ggc atg ctt gag atg atc aac ttc tcc   864
Lys Ala Ala Ile Met Arg Arg Gly Met Leu Glu Met Ile Asn Phe Ser
        275                 280                 285
aag cga ttc ttc ccc gaa act gat att aac act ctt aca gtc gag tct   912
Lys Arg Phe Phe Pro Glu Thr Asp Ile Asn Thr Leu Thr Val Glu Ser
    290                 295                 300
gcc ggt gtg gcc gat ctc atc acc tcg tgc gct gga ggc cga aac ttc   960
Ala Gly Val Ala Asp Leu Ile Thr Ser Cys Ala Gly Gly Arg Asn Phe
305                 310                 315                 320
aag gtc ggc cga gca ttc gga aag gag agc ggc tcc ggc aag acc atc  1008
Lys Val Gly Arg Ala Phe Gly Lys Glu Ser Gly Ser Gly Lys Thr Ile
                325                 330                 335
cag gac gtg gag aag gag ctt ctc aac ggc cag tcc gcc cag ggc gtc  1056
Gln Asp Val Glu Lys Glu Leu Leu Asn Gly Gln Ser Ala Gln Gly Val
            340                 345                 350
atc aca tgt aac gag gtc cac gag ctg ctc aag aac aag aac atg cag  1104
Ile Thr Cys Asn Glu Val His Glu Leu Leu Lys Asn Lys Asn Met Gln
        355                 360                 365
```

aag gac ttc cct ctg ttc gag tcc acc tgg ggc att atc cac ggt gag 1152
Lys Asp Phe Pro Leu Phe Glu Ser Thr Trp Gly Ile Ile His Gly Glu
370 375 380

ctc aag att gat gat ctc ccc gag att ctt tac cac gcc aac tag 1197
Leu Lys Ile Asp Asp Leu Pro Glu Ile Leu Tyr His Ala Asn
385 390 395

<210> 11
<211> 398
<212> PRT
<213> Yarrowia lipolytica

<400> 11
Met Ser Ala Leu Leu Arg Ser Ser Leu Arg Phe Lys His Met Ser Ala
1 5 10 15
Val Asn Arg Leu Thr Gln Gln Leu Arg Leu Leu Thr Ala Ser Ala Pro
20 25 30
Leu Ser Ala Ala Asn Thr Ala Gly Lys Ala Pro Phe Lys Val Ala Val
35 40 45
Val Gly Ser Gly Asn Trp Gly Thr Thr Val Ala Lys Ile Val Ala Glu
50 55 60
Asn Cys Thr Ala His Pro Glu Leu Phe Glu Pro Glu Val Arg Val Trp
65 70 75 80
Val Arg Glu Glu Lys Val Asn Gly Lys Asn Leu Thr Asp Ile Phe Asn
85 90 95
Ala Glu His Glu Asn Val Arg Tyr Leu Pro Lys Ile Lys Leu Pro His
100 105 110
Asn Leu Ile Ala Glu Pro Asp Leu Leu Lys Ala Val Glu Gly Ala Asn
115 120 125
Ile Ile Val Phe Asn Leu Pro His Gln Phe Leu Ala Gly Val Cys Lys
130 135 140
Gln Leu Lys Gly His Val Asn Pro Lys Ala Arg Ala Ile Ser Cys Leu
145 150 155 160
Lys Gly Leu Asp Val Thr Pro Gln Gly Val Tyr Leu Leu Ser Asp Val
165 170 175
Ile Glu Asn Glu Thr Gly Leu His Cys Gly Val Leu Ser Gly Ala Asn
180 185 190
Leu Ala Thr Glu Ile Ala Leu Glu Lys Tyr Ser Glu Thr Thr Val Ala
195 200 205
Tyr Asn Arg Pro Lys Asp Phe Phe Gly Glu Gly Asp Val Thr Asn Asp
210 215 220
Val Leu Lys Ala Leu Phe His Arg Pro Tyr Phe His Val Arg Cys Val
225 230 235 240
Gln Asp Val Ala Gly Val Ser Ile Gly Gly Ala Leu Lys Asn Val Val
245 250 255
Ala Leu Cys Ala Gly Phe Val Glu Gly Lys Asn Trp Gly Asp Asn Ala
260 265 270
Lys Ala Ala Ile Met Arg Arg Gly Met Leu Glu Met Ile Asn Phe Ser

```
        275                 280                 285
Lys Arg Phe Phe Pro Glu Thr Asp Ile Asn Thr Leu Thr Val Glu Ser
    290                 295                 300
Ala Gly Val Ala Asp Leu Ile Thr Ser Cys Ala Gly Gly Arg Asn Phe
305                 310                 315                 320
Lys Val Gly Arg Ala Phe Gly Lys Glu Ser Gly Ser Gly Lys Thr Ile
                325                 330                 335
Gln Asp Val Glu Lys Glu Leu Leu Asn Gly Gln Ser Ala Gln Gly Val
            340                 345                 350
Ile Thr Cys Asn Glu Val His Glu Leu Leu Lys Asn Lys Asn Met Gln
        355                 360                 365
Lys Asp Phe Pro Leu Phe Glu Ser Thr Trp Gly Ile Ile His Gly Glu
    370                 375                 380
Leu Lys Ile Asp Asp Leu Pro Glu Ile Leu Tyr His Ala Asn
385                 390                 395
```

<210> 12
<211> 385
<212> PRT
<213> Yarrowia lipolytica

<400> 12

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Met Ser Ala Val Asn Arg Leu Thr Gln Gln Leu Arg Leu Leu Thr Ala
  1               5                  10                  15
Ser Ala Pro Leu Ser Ala Ala Asn Thr Ala Gly Lys Ala Pro Phe Lys
             20                  25                  30
Val Ala Val Val Gly Ser Gly Asn Trp Gly Thr Thr Val Ala Lys Ile
         35                  40                  45
Val Ala Glu Asn Cys Thr Ala His Pro Glu Leu Phe Glu Pro Glu Val
     50                  55                  60
Arg Val Trp Val Arg Glu Glu Lys Val Asn Gly Lys Asn Leu Thr Asp
 65                  70                  75                  80
Ile Phe Asn Ala Glu His Glu Asn Val Arg Tyr Leu Pro Lys Ile Lys
                 85                  90                  95
Leu Pro His Asn Leu Ile Ala Glu Pro Asp Leu Leu Lys Ala Val Glu
            100                 105                 110
Gly Ala Asn Ile Ile Val Phe Asn Leu Pro His Gln Phe Leu Ala Gly
        115                 120                 125
Val Cys Lys Gln Leu Lys Gly His Val Asn Pro Lys Ala Arg Ala Ile
    130                 135                 140
Ser Cys Leu Lys Gly Leu Asp Val Thr Pro Gln Gly Val Tyr Leu Leu
145                 150                 155                 160
Ser Asp Val Ile Glu Asn Glu Thr Gly Leu His Cys Gly Val Leu Ser
                165                 170                 175
Gly Ala Asn Leu Ala Thr Glu Ile Ala Leu Glu Lys Tyr Ser Glu Thr
            180                 185                 190
Thr Val Ala Tyr Asn Arg Pro Lys Asp Phe Phe Gly Glu Gly Asp Val
        195                 200                 205
```

Thr Asn Asp Val Leu Lys Ala Leu Phe His Arg Pro Tyr Phe His Val
210 215 220
Arg Cys Val Gln Asp Val Ala Gly Val Ser Ile Gly Gly Ala Leu Lys
225 230 235 240
Asn Val Val Ala Leu Cys Ala Gly Phe Val Glu Gly Lys Asn Trp Gly
245 250 255
Asp Asn Ala Lys Ala Ala Ile Met Arg Arg Gly Met Leu Glu Met Ile
260 265 270
Asn Phe Ser Lys Arg Phe Phe Pro Glu Thr Asp Ile Asn Thr Leu Thr
275 280 285
Val Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Ser Cys Ala Gly Gly
290 295 300
Arg Asn Phe Lys Val Gly Arg Ala Phe Gly Lys Glu Ser Gly Ser Gly
305 310 315 320
Lys Thr Ile Gln Asp Val Glu Lys Glu Leu Leu Asn Gly Gln Ser Ala
325 330 335
Gln Gly Val Ile Thr Cys Asn Glu Val His Glu Leu Leu Lys Asn Lys
340 345 350
Asn Met Gln Lys Asp Phe Pro Leu Phe Glu Ser Thr Trp Gly Ile Ile
355 360 365
His Gly Glu Leu Lys Ile Asp Asp Leu Pro Glu Ile Leu Tyr His Ala
370 375 380
Asn
385

<210> 13
<211> 1206
<212> DNA
<213> Zygosaccharomyces rouxii

<220>
<221> CDS
<222> (1)..(1203)
<223> coding for G3PDH

<400> 13
atg gcc gct act gac aga tta aac caa acc tct gat atc cta tcg caa 48
Met Ala Ala Thr Asp Arg Leu Asn Gln Thr Ser Asp Ile Leu Ser Gln
1 5 10 15
tct atg aag aag acc gac tca tca atg tca gtc gtt acc gct gag aat 96
Ser Met Lys Lys Thr Asp Ser Ser Met Ser Val Val Thr Ala Glu Asn
20 25 30
cca tac aaa gtt tcc gtc gtc ggc tct ggt aac tgg ggt acc acc atc 144
Pro Tyr Lys Val Ser Val Val Gly Ser Gly Asn Trp Gly Thr Thr Ile
35 40 45
gcc aag gtc gtt gcc gaa aac acc aag gaa aag cca gaa ttg ttc caa 192
Ala Lys Val Val Ala Glu Asn Thr Lys Glu Lys Pro Glu Leu Phe Gln
50 55 60

```
gaa cgt gtg gac atg tgg gtg ttt gaa gaa cag atc gac ggt act cca   240
Glu Arg Val Asp Met Trp Val Phe Glu Glu Gln Ile Asp Gly Thr Pro
 65                  70                  75                  80
ttg gcc caa atc atc aac acc aag cac cag aac gtg aaa tac ttg cca   288
Leu Ala Gln Ile Ile Asn Thr Lys His Gln Asn Val Lys Tyr Leu Pro
                 85                  90                  95
aac atc gac ctt ccg gac aat ttg gtc gct aac cca gac ttg att gcc   336
Asn Ile Asp Leu Pro Asp Asn Leu Val Ala Asn Pro Asp Leu Ile Ala
            100                 105                 110
acc acg aag gac gcc gat gtg att gtt ttc aac gtt ccc cat caa ttt   384
Thr Thr Lys Asp Ala Asp Val Ile Val Phe Asn Val Pro His Gln Phe
        115                 120                 125
ttg ggc cgt atc gtt gct caa atg aag ggt caa atc aaa cca act gca   432
Leu Gly Arg Ile Val Ala Gln Met Lys Gly Gln Ile Lys Pro Thr Ala
    130                 135                 140
cgt gcg gtc tcc tgt cta aag ggt ttc gaa gtt ggt cca aag ggt gtg   480
Arg Ala Val Ser Cys Leu Lys Gly Phe Glu Val Gly Pro Lys Gly Val
145                 150                 155                 160
cag ctt cta tct gac tac gtc act caa gaa ttg ggt atc gaa tgt ggt   528
Gln Leu Leu Ser Asp Tyr Val Thr Gln Glu Leu Gly Ile Glu Cys Gly
                165                 170                 175
gct cta tct ggt gct aac ttg gcc cca gaa gtc gcc aag gaa cac tgg   576
Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu His Trp
            180                 185                 190
tcc gag acc acc gtc gct tac cac atc cca gac gac ttc aag ggt gac   624
Ser Glu Thr Thr Val Ala Tyr His Ile Pro Asp Asp Phe Lys Gly Asp
        195                 200                 205
ggt aag gac atc gac cac cgt gtc ttg aag cag ttg ttc cac aga cca   672
Gly Lys Asp Ile Asp His Arg Val Leu Lys Gln Leu Phe His Arg Pro
    210                 215                 220
tac ttc cac gtg aat gtg att gac gat gtt gct ggt atc tcc atc gca   720
Tyr Phe His Val Asn Val Ile Asp Asp Val Ala Gly Ile Ser Ile Ala
225                 230                 235                 240
ggt gca ttg aag aac gtg gtc gcc ttg ggt tgc ggt ttc gtt acc ggt   768
Gly Ala Leu Lys Asn Val Val Ala Leu Gly Cys Gly Phe Val Thr Gly
                245                 250                 255
cta ggt tgg ggt aac aac gcc gcc gcc gcc atc caa cgt gtc ggt ttg   816
Leu Gly Trp Gly Asn Asn Ala Ala Ala Ala Ile Gln Arg Val Gly Leu
            260                 265                 270
ggt gaa atc atc aag ttc ggt agg atg ttc ttc cca gaa tcc aag gtg   864
Gly Glu Ile Ile Lys Phe Gly Arg Met Phe Phe Pro Glu Ser Lys Val
        275                 280                 285
gag act tac tac caa gaa tcc gca ggt gtt gct gac ttg atc acc acc   912
Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Thr
    290                 295                 300
```

```
tgt tcc ggt ggt aga aac gtc cgt gtt gcc acc gaa atg gcc aag act   960
Cys Ser Gly Gly Arg Asn Val Arg Val Ala Thr Glu Met Ala Lys Thr
305                 310                 315                 320
ggt aag agc ggt gag caa gtc gaa aaa gac atc ttg aac ggt caa tcc   1008
Gly Lys Ser Gly Glu Gln Val Glu Lys Asp Ile Leu Asn Gly Gln Ser
                325                 330                 335
gct caa ggt ttg gtc acc tgt aag gaa gtt cac cag tgg tta gaa tct   1056
Ala Gln Gly Leu Val Thr Cys Lys Glu Val His Gln Trp Leu Glu Ser
            340                 345                 350
agt gga aac acc gaa gac ttc cca ttg ttc gag gct gtc tac cag atc   1104
Ser Gly Asn Thr Glu Asp Phe Pro Leu Phe Glu Ala Val Tyr Gln Ile
        355                 360                 365
act tac gaa aac gtg ccc atg aag gag ttg cca tct atg atc gaa gaa   1152
Thr Tyr Glu Asn Val Pro Met Lys Glu Leu Pro Ser Met Ile Glu Glu
    370                 375                 380
ttg gat atc gat agc aca tcg aag tgc gta ttg agt tac aag atg ggt   1200
Leu Asp Ile Asp Ser Thr Ser Lys Cys Val Leu Ser Tyr Lys Met Gly
385                 390                 395                 400
ctc tag                                                          1206
Leu
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<210> 14
<211> 401
<212> PRT
<213> Zygosaccharomyces rouxii

<400> 14

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Met Ala Ala Thr Asp Arg Leu Asn Gln Thr Ser Asp Ile Leu Ser Gln
  1               5                  10                  15
Ser Met Lys Lys Thr Asp Ser Ser Met Ser Val Val Thr Ala Glu Asn
            20                  25                  30
Pro Tyr Lys Val Ser Val Val Gly Ser Gly Asn Trp Gly Thr Thr Ile
        35                  40                  45
Ala Lys Val Val Ala Glu Asn Thr Lys Glu Lys Pro Glu Leu Phe Gln
    50                  55                  60
Glu Arg Val Asp Met Trp Val Phe Glu Glu Gln Ile Asp Gly Thr Pro
65                  70                  75                  80
Leu Ala Gln Ile Ile Asn Thr Lys His Gln Asn Val Lys Tyr Leu Pro
                85                  90                  95
Asn Ile Asp Leu Pro Asp Asn Leu Val Ala Asn Pro Asp Leu Ile Ala
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Thr Thr Lys Asp Ala Asp Val Ile Val Phe Asn Val Pro His Gln Phe
        115                 120                 125
Leu Gly Arg Ile Val Ala Gln Met Lys Gly Gln Ile Lys Pro Thr Ala
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Arg Ala Val Ser Cys Leu Lys Gly Phe Glu Val Gly Pro Lys Gly Val
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Gln Leu Leu Ser Asp Tyr Val Thr Gln Glu Leu Gly Ile Glu Cys Gly
165 170 175

Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu His Trp
180 185 190

Ser Glu Thr Thr Val Ala Tyr His Ile Pro Asp Asp Phe Lys Gly Asp
195 200 205

Gly Lys Asp Ile Asp His Arg Val Leu Lys Gln Leu Phe His Arg Pro
210 215 220

Tyr Phe His Val Asn Val Ile Asp Asp Val Ala Gly Ile Ser Ile Ala
225 230 235 240

Gly Ala Leu Lys Asn Val Val Ala Leu Gly Cys Gly Phe Val Thr Gly
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Leu Gly Trp Gly Asn Asn Ala Ala Ala Ala Ile Gln Arg Val Gly Leu
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Gly Glu Ile Ile Lys Phe Gly Arg Met Phe Phe Pro Glu Ser Lys Val
275 280 285

Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Thr
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Cys Ser Gly Gly Arg Asn Val Arg Val Ala Thr Glu Met Ala Lys Thr
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Gly Lys Ser Gly Glu Gln Val Glu Lys Asp Ile Leu Asn Gly Gln Ser
325 330 335

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Ser Gly Asn Thr Glu Asp Phe Pro Leu Phe Glu Ala Val Tyr Gln Ile
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Ser Met Lys Lys Thr Asp Thr Ser Met Ser Ile Val Thr Ala Glu Asn
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cct tac aag gtc gct gtt gtc ggt tct ggt aac tgg ggt acc act atc 144
Pro Tyr Lys Val Ala Val Val Gly Ser Gly Asn Trp Gly Thr Thr Ile
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gct aag gtt gtt gcc gaa aac acc aaa gaa aag cca gag ttg ttc caa 192
Ala Lys Val Val Ala Glu Asn Thr Lys Glu Lys Pro Glu Leu Phe Gln
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Gly Arg Val Asp Met Trp Val Phe Glu Glu Gln Ile Asp Gly Thr Pro
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Leu Thr Gln Ile Ile Asn Thr Lys His Gln Asn Val Lys Tyr Leu Pro
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Asn Ile Asp Leu Pro Gly Asn Leu Val Ala Asn Pro Asp Leu Ile Ser
100 105 110

act acc aag gac gct gat gtc atc gtt ttc aac gtt cct cac caa ttt 384
Thr Thr Lys Asp Ala Asp Val Ile Val Phe Asn Val Pro His Gln Phe
115 120 125

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Leu Gly Arg Ile Val Ser Gln Met Lys Gly Gln Ile Lys Pro Asp Ala
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Arg Ala Ile Ser Cys Leu Lys Gly Phe Glu Val Gly Pro Lys Gly Val
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caa cta ctt tct gac tac gtc act caa gaa tta ggt atc caa tgt ggt 528
Gln Leu Leu Ser Asp Tyr Val Thr Gln Glu Leu Gly Ile Gln Cys Gly
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gcc cta tct ggt gct aac ttg gct cca gaa gtc gcc aag gaa cac tgg 576
Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu His Trp
180 185 190

tcc gaa act acc gtc gct tac caa gtc cca gat gac ttc aag ggt gaa 624
Ser Glu Thr Thr Val Ala Tyr Gln Val Pro Asp Asp Phe Lys Gly Glu
195 200 205

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Gly Lys Asp Ile Asp His Arg Val Leu Lys Gln Leu Phe His Arg Pro
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Tyr Phe His Val Asn Val Ile Asp Asp Val Ala Gly Ile Ser Ile Ala
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ggt gca ttg aag aac gtg gtt gcc ttg ggt tgc ggt ttc gtc acc ggt   768
Gly Ala Leu Lys Asn Val Val Ala Leu Gly Cys Gly Phe Val Thr Gly
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cta ggc tgg ggt aac aac gct gcc gcc gcc atc caa cgt gtt ggt ttg   816
Leu Gly Trp Gly Asn Asn Ala Ala Ala Ala Ile Gln Arg Val Gly Leu
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Gly Glu Ile Ile Lys Phe Gly Arg Met Phe Phe Pro Glu Ser Lys Val
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gaa act tac tac caa gaa tct gca ggt gtt gct gat ttg atc act acc   912
Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Thr
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tgt tcc ggt ggt aga aac gtt cgt gtc gcc act gaa atg gcc aag act   960
Cys Ser Gly Gly Arg Asn Val Arg Val Ala Thr Glu Met Ala Lys Thr
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ggt aag agc ggt gaa caa gtc gaa aag gac atc ttg aac ggt caa tcc  1008
Gly Lys Ser Gly Glu Gln Val Glu Lys Asp Ile Leu Asn Gly Gln Ser
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act tac gaa aac gtg ccc atg aag gag ttg cca tcc atg atc gaa gaa  1152
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Pro Tyr Lys Val Ala Val Val Gly Ser Gly Asn Trp Gly Thr Thr Ile
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Asn Ile Asp Leu Pro Gly Asn Leu Val Ala Asn Pro Asp Leu Ile Ser
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Thr Thr Lys Asp Ala Asp Val Ile Val Phe Asn Val Pro His Gln Phe
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Gln Leu Leu Ser Asp Tyr Val Thr Gln Glu Leu Gly Ile Gln Cys Gly
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Ala Leu Ser Gly Ala Asn Leu Ala Pro Glu Val Ala Lys Glu His Trp
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Ser Glu Thr Thr Val Ala Tyr Gln Val Pro Asp Asp Phe Lys Gly Glu
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Glu Thr Tyr Tyr Gln Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Thr
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<220>
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sequence motive

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<220>
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sequence motive

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<223> Ile or Val
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<223> Ile

<400> 25
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<223> Description of the artificial sequence: Yeast G3PDH sequence motive

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<223> Ile or Val

<220>
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gga tcg ggt aac tgg ggc acc gct ata gcc aaa atc gtc gcc gag aat   96
Gly Ser Gly Asn Trp Gly Thr Ala Ile Ala Lys Ile Val Ala Glu Asn
            20                  25                  30

act gcc agc aac cct gcg gtc ttt gag aag gat gtt cag atg tgg gtt   144
Thr Ala Ser Asn Pro Ala Val Phe Glu Lys Asp Val Gln Met Trp Val
        35                  40                  45

ttc gag gaa aag gtc gag att ccg aaa tcg tcg aag cat tat gat cct   192
Phe Glu Glu Lys Val Glu Ile Pro Lys Ser Ser Lys His Tyr Asp Pro
    50                  55                  60

gcc tct tct ctt tgc cag ggc ccg cag aat ctg aca gat att atc aac   240
Ala Ser Ser Leu Cys Gln Gly Pro Gln Asn Leu Thr Asp Ile Ile Asn
65                  70                  75                  80

cat acc cat gag aat atc aag tac ctc ccc gga att acc ctt ccg gaa   288
His Thr His Glu Asn Ile Lys Tyr Leu Pro Gly Ile Thr Leu Pro Glu
                85                  90                  95
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aac ttg att gcc aat cca tcg cta gtc gac gcg gtt caa gac agc act   336
Asn Leu Ile Ala Asn Pro Ser Leu Val Asp Ala Val Gln Asp Ser Thr
            100                 105                 110

atc ctc gtc ttc aac cta ccc cat caa ttc atc atc aat att tgt gaa   384
Ile Leu Val Phe Asn Leu Pro His Gln Phe Ile Ile Asn Ile Cys Glu
        115                 120                 125

cag atc aag ggc aag att gtc cca tac gcg cgt gga att tct tgc ata   432
Gln Ile Lys Gly Lys Ile Val Pro Tyr Ala Arg Gly Ile Ser Cys Ile
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aag ggc gtg gat gtg aat gag gaa gga gtc cac ctg ttt tcc gaa aca   480
Lys Gly Val Asp Val Asn Glu Glu Gly Val His Leu Phe Ser Glu Thr
145                 150                 155                 160

att gga aag att ctc ggg atc tac tgt ggc gcc ctg tcc ggt gcc aac   528
Ile Gly Lys Ile Leu Gly Ile Tyr Cys Gly Ala Leu Ser Gly Ala Asn
                165                 170                 175

atc gcg aat gag gtc gcc cag gaa aag tgg tcc gag tct agc att ggt   576
Ile Ala Asn Glu Val Ala Gln Glu Lys Trp Ser Glu Ser Ser Ile Gly
            180                 185                 190

tat gat cca ccg cat ttt gac tct aaa gcc cct tct cct ccc aac cga   624
Tyr Asp Pro Pro His Phe Asp Ser Lys Ala Pro Ser Pro Pro Asn Arg
        195                 200                 205

tcc cct tcc gca tcg act gac aat atc ctg cac ttc gag cac aaa gac   672
Ser Pro Ser Ala Ser Thr Asp Asn Ile Leu His Phe Glu His Lys Asp
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gtt tcg ggt caa ctt tcg cgg gta aag cta cag gct cta cct tcc gaa   720
Val Ser Gly Gln Leu Ser Arg Val Lys Leu Gln Ala Leu Pro Ser Glu
225                 230                 235                 240

ttt cct ccc atc gac cat gcc ctt ctc aag tcg cta ttc cac cgt cct   768
Phe Pro Pro Ile Asp His Ala Leu Leu Lys Ser Leu Phe His Arg Pro
                245                 250                 255

tac ttc cat att ggt gtg gta agt gac gtc gca ggt gtt tcg tta gga   816
Tyr Phe His Ile Gly Val Val Ser Asp Val Ala Gly Val Ser Leu Gly
            260                 265                 270

ggt gcc ctt aag aat gtc gtt gct gtc gcg gca ggg tgg gtt gtg ggc   864
Gly Ala Leu Lys Asn Val Val Ala Val Ala Ala Gly Trp Val Val Gly
        275                 280                 285

aaa gga tgg gga gac aat gcg aag gct gca att atg cga gtt ggg ctt   912
Lys Gly Trp Gly Asp Asn Ala Lys Ala Ala Ile Met Arg Val Gly Leu
    290                 295                 300

ttg gaa atg gtg aag ttc ggc gaa cag ttt ttc ggt gct acc atc aac   960
Leu Glu Met Val Lys Phe Gly Glu Gln Phe Phe Gly Ala Thr Ile Asn
305                 310                 315                 320

act cgc acc ttc act gaa gaa agt gct ggt gtt gcc gat cta atc acg  1008
Thr Arg Thr Phe Thr Glu Glu Ser Ala Gly Val Ala Asp Leu Ile Thr
                325                 330                 335
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Ser Cys Ser Gly Gly Arg Asn Phe Arg Cys Ala Lys Leu Ser Ile Glu
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aga aac cag ccg att gag aaa atc gag gag aca gag ttg aac ggc cag 1104
Arg Asn Gln Pro Ile Glu Lys Ile Glu Glu Thr Glu Leu Asn Gly Gln
355 360 365

aag ctg caa ggc act ttg act gca gtc gaa gtc aac agt ttc ttg aaa 1152
Lys Leu Gln Gly Thr Leu Thr Ala Val Glu Val Asn Ser Phe Leu Lys
370 375 380

aag caa ggt tta gaa gaa gag ttc cca ttg ttt act gca gtc tac cga 1200
Lys Gln Gly Leu Glu Glu Glu Phe Pro Leu Phe Thr Ala Val Tyr Arg
385 390 395 400

gtt ctt caa ggc acc atg tct gtg gac gag att cct tct ttc att gag 1248
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35 40 45
Phe Glu Glu Lys Val Glu Ile Pro Lys Ser Ser Lys His Tyr Asp Pro
50 55 60
Ala Ser Ser Leu Cys Gln Gly Pro Gln Asn Leu Thr Asp Ile Ile Asn
65 70 75 80
His Thr His Glu Asn Ile Lys Tyr Leu Pro Gly Ile Thr Leu Pro Glu
85 90 95
Asn Leu Ile Ala Asn Pro Ser Leu Val Asp Ala Val Gln Asp Ser Thr
100 105 110
Ile Leu Val Phe Asn Leu Pro His Gln Phe Ile Ile Asn Ile Cys Glu
115 120 125
Gln Ile Lys Gly Lys Ile Val Pro Tyr Ala Arg Gly Ile Ser Cys Ile
130 135 140
Lys Gly Val Asp Val Asn Glu Glu Gly Val His Leu Phe Ser Glu Thr
145 150 155 160
Ile Gly Lys Ile Leu Gly Ile Tyr Cys Gly Ala Leu Ser Gly Ala Asn
165 170 175

Ile Ala Asn Glu Val Ala Gln Glu Lys Trp Ser Glu Ser Ser Ile Gly
180 185 190

Tyr Asp Pro Pro His Phe Asp Ser Lys Ala Pro Ser Pro Pro Asn Arg
195 200 205

Ser Pro Ser Ala Ser Thr Asp Asn Ile Leu His Phe Glu His Lys Asp
210 215 220

Val Ser Gly Gln Leu Ser Arg Val Lys Leu Gln Ala Leu Pro Ser Glu
225 230 235 240

Phe Pro Pro Ile Asp His Ala Leu Leu Lys Ser Leu Phe His Arg Pro
245 250 255

Tyr Phe His Ile Gly Val Val Ser Asp Val Ala Gly Val Ser Leu Gly
260 265 270

Gly Ala Leu Lys Asn Val Val Ala Val Ala Ala Gly Trp Val Val Gly
275 280 285

Lys Gly Trp Gly Asp Asn Ala Lys Ala Ala Ile Met Arg Val Gly Leu
290 295 300

Leu Glu Met Val Lys Phe Gly Glu Gln Phe Phe Gly Ala Thr Ile Asn
305 310 315 320

Thr Arg Thr Phe Thr Glu Glu Ser Ala Gly Val Ala Asp Leu Ile Thr
325 330 335

Ser Cys Ser Gly Gly Arg Asn Phe Arg Cys Ala Lys Leu Ser Ile Glu
340 345 350

Arg Asn Gln Pro Ile Glu Lys Ile Glu Glu Thr Glu Leu Asn Gly Gln
355 360 365

Lys Leu Gln Gly Thr Leu Thr Ala Val Glu Val Asn Ser Phe Leu Lys
370 375 380

Lys Gln Gly Leu Glu Glu Glu Phe Pro Leu Phe Thr Ala Val Tyr Arg
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Val Leu Gln Gly Thr Met Ser Val Asp Glu Ile Pro Ser Phe Ile Glu
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Arg

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 1               5                  10                  15

acg gct gaa aaa cca gaa gtg ttc gaa aag caa gtg aac atg tgg gtt   96
Thr Ala Glu Lys Pro Glu Val Phe Glu Lys Gln Val Asn Met Trp Val
            20                  25                  30

ttt gaa gaa gaa gtt gac gga caa aag ttg act gaa atc atc aac gcc  144
Phe Glu Glu Glu Val Asp Gly Gln Lys Leu Thr Glu Ile Ile Asn Ala
        35                  40                  45

aaa cac gaa aac gtt aag tac ttg cca gaa gtc aag ttg ccg gaa aac  192
Lys His Glu Asn Val Lys Tyr Leu Pro Glu Val Lys Leu Pro Glu Asn
    50                  55                  60

ttg gtt gca aac cca gac gtt gtt gac act gtc aag gat gca gac tta  240
Leu Val Ala Asn Pro Asp Val Val Asp Thr Val Lys Asp Ala Asp Leu
65                  70                  75                  80

tta att ttt aac att cca cat caa ttc tta cca aga gtg tgt aag caa  288
Leu Ile Phe Asn Ile Pro His Gln Phe Leu Pro Arg Val Cys Lys Gln
                85                  90                  95

ttg gtt ggc cat gtc aag cca tct gcc aga gcc atc tcc tgt ttg aag  336
Leu Val Gly His Val Lys Pro Ser Ala Arg Ala Ile Ser Cys Leu Lys
            100                 105                 110

ggt ttg gaa gtt ggc cca gaa ggt tgt aag ttg tta tcg caa tct atc  384
Gly Leu Glu Val Gly Pro Glu Gly Cys Lys Leu Leu Ser Gln Ser Ile
        115                 120                 125

aac gat act tta ggt gtc cac tgt ggt gtc tta tct ggt gcc aac att  432
Asn Asp Thr Leu Gly Val His Cys Gly Val Leu Ser Gly Ala Asn Ile
    130                 135                 140

gcc aac gaa gtt gcc aga gaa aga tgg tct gaa acc acc att gcc tac  480
Ala Asn Glu Val Ala Arg Glu Arg Trp Ser Glu Thr Thr Ile Ala Tyr
145                 150                 155                 160

aac att cca gaa gat ttc aga ggt aag ggt aga gat atc gac gaa tac  528
Asn Ile Pro Glu Asp Phe Arg Gly Lys Gly Arg Asp Ile Asp Glu Tyr
                165                 170                 175

gtc tta aag caa tta ttc cac aga acc tac ttc cat gtc aga gtc atc  576
Val Leu Lys Gln Leu Phe His Arg Thr Tyr Phe His Val Arg Val Ile
            180                 185                 190

aac gac atc ata ggt gct tct ttc gct ggt gct ttg aag aat gtt gtt  624
Asn Asp Ile Ile Gly Ala Ser Phe Ala Gly Ala Leu Lys Asn Val Val
        195                 200                 205

gcc tgt gct gtt ggt ttc gtt atc ggt gcc ggc tgg ggt gac aac gct  672
Ala Cys Ala Val Gly Phe Val Ile Gly Ala Gly Trp Gly Asp Asn Ala
    210                 215                 220
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aag gcc gct atc atg aga atc ggt atc aga gaa atc atc cac ttt gcc   720
Lys Ala Ala Ile Met Arg Ile Gly Ile Arg Glu Ile Ile His Phe Ala
225                 230                 235                 240

tct tac tac caa aag ttc ggt gtc aag ggt cca gct cca gaa tcc act   768
Ser Tyr Tyr Gln Lys Phe Gly Val Lys Gly Pro Ala Pro Glu Ser Thr
                245                 250                 255

act ttc act gag gaa tct gcc ggt gtc gct gac tta atc acc act tgt   816
Thr Phe Thr Glu Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Thr Cys
            260                 265                 270

tcc ggt ggt aga aat gtc aag gtt gct aga tac atg att gaa aac aac   864
Ser Gly Gly Arg Asn Val Lys Val Ala Arg Tyr Met Ile Glu Asn Asn
        275                 280                 285

gtt gac gct tgg gaa gcc gaa aag att gtc tta aag ggt caa tct tct   912
Val Asp Ala Trp Glu Ala Glu Lys Ile Val Leu Lys Gly Gln Ser Ser
    290                 295                 300

caa ggt atc tta act gcc aag gaa gtc cac gaa ttg tta act aac tac   960
Gln Gly Ile Leu Thr Ala Lys Glu Val His Glu Leu Leu Thr Asn Tyr
305                 310                 315                 320

aac tta tcg aat gaa ttc cca tta ttt gaa gcc gta tac               999
Asn Leu Ser Asn Glu Phe Pro Leu Phe Glu Ala Val
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<400> 40

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  1               5                  10                  15

Thr Ala Glu Lys Pro Glu Val Phe Glu Lys Gln Val Asn Met Trp Val
             20                  25                  30

Phe Glu Glu Glu Val Asp Gly Gln Lys Leu Thr Glu Ile Ile Asn Ala
         35                  40                  45

Lys His Glu Asn Val Lys Tyr Leu Pro Glu Val Lys Leu Pro Glu Asn
     50                  55                  60

Leu Val Ala Asn Pro Asp Val Val Asp Thr Val Lys Asp Ala Asp Leu
 65                  70                  75                  80

Leu Ile Phe Asn Ile Pro His Gln Phe Leu Pro Arg Val Cys Lys Gln
                 85                  90                  95

Leu Val Gly His Val Lys Pro Ser Ala Arg Ala Ile Ser Cys Leu Lys
            100                 105                 110

Gly Leu Glu Val Gly Pro Glu Gly Cys Lys Leu Leu Ser Gln Ser Ile
        115                 120                 125

Asn Asp Thr Leu Gly Val His Cys Gly Val Leu Ser Gly Ala Asn Ile
    130                 135                 140
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Ala Asn Glu Val Ala Arg Glu Arg Trp Ser Glu Thr Thr Ile Ala Tyr
145 150 155 160
Asn Ile Pro Glu Asp Phe Arg Gly Lys Gly Arg Asp Ile Asp Glu Tyr
165 170 175
Val Leu Lys Gln Leu Phe His Arg Thr Tyr Phe His Val Arg Val Ile
180 185 190
Asn Asp Ile Ile Gly Ala Ser Phe Ala Gly Ala Leu Lys Asn Val Val
195 200 205
Ala Cys Ala Val Gly Phe Val Ile Gly Ala Gly Trp Gly Asp Asn Ala
210 215 220
Lys Ala Ala Ile Met Arg Ile Gly Ile Arg Glu Ile Ile His Phe Ala
225 230 235 240
Ser Tyr Tyr Gln Lys Phe Gly Val Lys Gly Pro Ala Pro Glu Ser Thr
245 250 255
Thr Phe Thr Glu Glu Ser Ala Gly Val Ala Asp Leu Ile Thr Thr Cys
260 265 270
Ser Gly Gly Arg Asn Val Lys Val Ala Arg Tyr Met Ile Glu Asn Asn
275 280 285
Val Asp Ala Trp Glu Ala Glu Lys Ile Val Leu Lys Gly Gln Ser Ser
290 295 300
Gln Gly Ile Leu Thr Ala Lys Glu Val His Glu Leu Leu Thr Asn Tyr
305 310 315 320
Asn Leu Ser Asn Glu Phe Pro Leu Phe Glu Ala Val
325 330

We claim:

1. A method of increasing the total oil content in a plant organism or a tissue, organ, part, cell or propagation material thereof, comprising

   a) the transgenic expression of yeast glycerol-3-phosphate dehydrogenase in said plant organism or in a tissue, organ, part, cell or propagation material thereof, and

   b) the selection of plant organisms in which - in contrast to or comparison with the starting organism - the total oil content in said plant organism or in a tissue, organ, part, cell or propagation material thereof is increased.

2. A method as claimed in claim 1, wherein the glycerol-3-phosphate dehydrogenase is derived from a yeast selected from the genera Cryptococcus, Torulopsis, Pityrosporum, Brettanomyces, Candida, Kloeckera, Trigonopsis, Trichosporon, Rhodotorul, Sporobolomyces, Bullera, Saccharomyces, Debaromyces, Lipomyces, Hansenula, Endomycopsis, Pichia and Hanseniaspora.

3. A method as claimed in claim 1 or 2, wherein the glycerol-3-phosphate dehydrogenase is derived from a yeast selected from the species Saccharomyces cerevisiae, Pichia pastoris, Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Zygosaccharomyces rouxii, Yarrowia lipolitica, Emericella nidulans, Aspergillus nidulans, Debaryomyces hansenii and Torulaspora hansenii.

4. A method as claimed in any of claims 1 to 3, wherein the glycerol-3-phosphate dehydrogenase brings about the conversion of dihydroxyacetone phosphate to glycerol-3-phosphate using NADH as cosubstrate and has a peptide sequence encompassing at least one sequence motif selected from the group of sequence motifs consisting of

   i) GSGNWGT(A/T)IAK
   ii) CG(V/A)LSGAN(L/I/V)AXE(V/I)A
   iii) (L/V)FXRPYFXV

5. A method as claimed in any of claims 1 to 4, wherein the glycerol-3-phosphate dehydrogenase brings about the conversion of dihydroxyacetone phosphate to glycerol-3-phosphate using NADH as cosubstrate and has a

peptide sequence encompassing at least one sequence motif selected from the group of sequence motifs consisting of

iv) GSGNWGTTIAKV(V/I)AEN
v) NT(K/R)HQNVKYLP
vi) D(I/V)LVFN(I/V)PHQFL
vii) RA(I/V)SCLKGFE
viii) CGALSGANLA(P/T)EVA
ix) LFHRPYFHV
x) GLGEII(K/R)FG

6. A method as claimed in claim 4 or 5, wherein the glycerol-3-phosphate dehydrogenase additionally encompasses at least one sequence motif selected from the group of sequence motifs consisting of

xi) H(E/Q)NVKYL
xii) (D/N)(I/V)(L/I)V(F/W)(V/N)(L/I/V)PHQF(V/L/I)
xiii) (A/G)(I/V)SC(L/I)KG
xiv) G(L/M)(L/G)E(M/I)(I/Q)(R/K/N)F(G/S/A)

7. A method as claimed in any of claims 1 to 6, wherein the yeast glycerol-3-phosphate dehydrogenase is described by

a) a sequence with the SEQ ID NO: 2, 4, 5, 7, 9, 11, 12, 14, 16, 38 or 40, or

b) a functional equivalent of a) with an identity of at least 60% of a sequence with SEQ ID NO: 2.

8. A method as claimed in any of claims 1 to 7, wherein the plant is an oil crop.

9. A method as claimed in any of claims 1 to 8, wherein the total oil content in the seed of a plant is increased.

10. A transgenic expression cassette comprising, under the control of a promoter which is functional in a plant organism or a tissue, organ, part or cell thereof, a nucleic acid sequence encoding a yeast glycerol-3-phosphate dehydrogenase as defined in any of claims 2 to 7.

11. A transgenic expression cassette as claimed in claim 10, wherein the nucleic acid sequence encoding a glycerol-3-phosphate dehydrogenase is described by

a) a sequence with the SEQ ID NO: 1, 3, 6, 8, 10, 13, 15, 37 or 39 or

b) a sequence derived from a sequence with the SEQ ID NO: 1, 3, 6, 8, 10, 13, 15, 37 or 39 in accordance with the degeneracy of the genetic code

c) a sequence which has at least 60% identity with the sequence with the SEQ ID NO: 1.

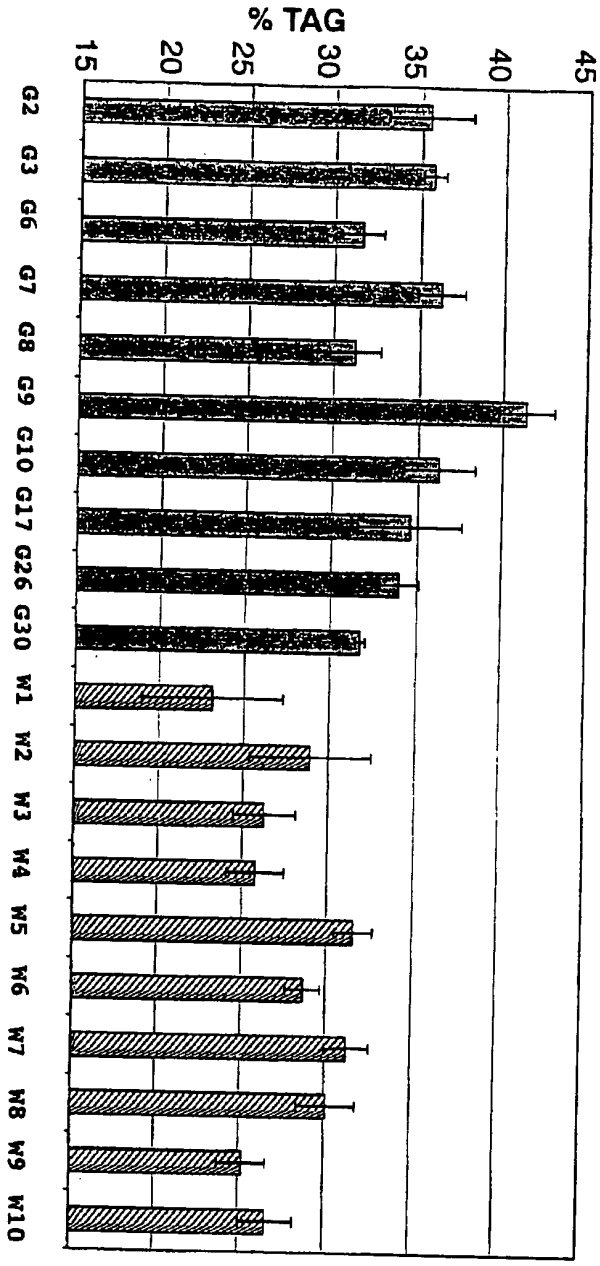
12. A transgenic expression cassette as claimed in claim 10 or 11, wherein the promoter is a seed-specific promotor.

13. A transgenic vector comprising an expression cassette as claimed in any of claims 10 to 12.

14. A transgenic plant organism or tissue, organ, part, cell or propagation material thereof, comprising a yeast glycerol-3-phosphate dehydrogenase as defined in any of claims 2 to 7 or an expression cassette as claimed in any of claims 10 to 12 or a vector as claimed in claim 13.

15. A transgenic plant organism as claimed in claim 14, wherein the plant organism is selected from the group of the oil crops consisting of Borvago officinalis, Brassica campestris, Brassica napus, Brassica rapa, Cannabis sativa, Carthamus tinctorius, Cocos nucifera, Crambe abyssinica, Cuphea species, Elaeis guinensis, Elaeis oleifera, Glycine max, Gossypium hirsutum, Gossypium barbadense, Gossypium herbaceum, Helianthus annuus, Linum usitatissimum, Oenothera biennis, Olea europaea, Oryza sativa, Ricinus communis, Sesamum indicum, Triticum species, Zea mays, walnut and almond.

16. The use of a transgenic plant organism or tissue, organ, part, cell or propagation material thereof as claimed in claim 14 or 15 for the production of oils, fats, free fatty acids or derivatives of the above.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G10
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W6
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N8
W9
W10

Fig.1

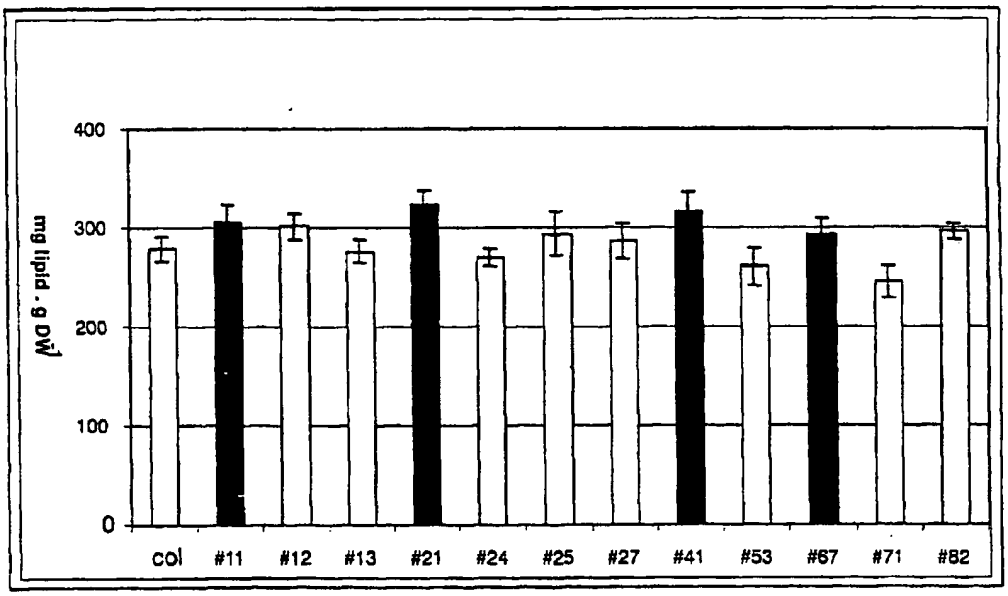
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Fig. 2

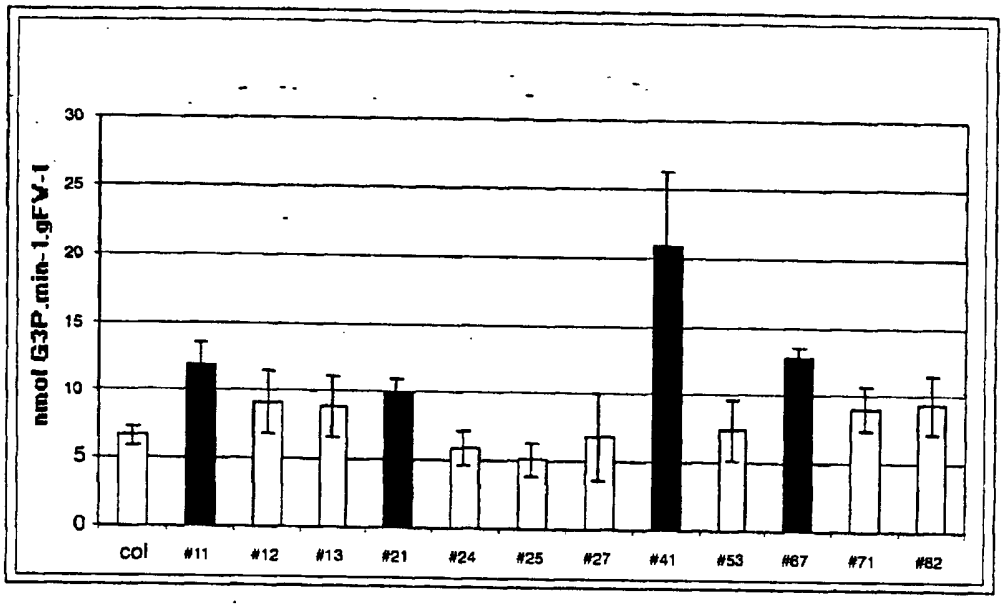
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Fig. 3